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EFFECT OF CHOLINERGIC STIMULATION ON SPONTANEOUS ADHESION OF LYMPHOCYTES IN VITRO

D. D. Kharkevich

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Potentiation of some immunologic reactions in the presence of cholinergic agonists [9] provided a basis for the hypothesis that acetylcholine receptors are present on the surface of lymphocytes. In most cases investigated the response to cholinergic drugs was depressed or completely abolished by atropine, a specific blocker of muscarinic acetylcholine (ACh) receptors. Direct investigations of binding of ^{3}H-quinuclidinyl benzylate, a specific ligand of muscarinic ACh receptors, showed that the reaction of lymphocytes to ACh is determined by the presence of muscarinic ACh receptors [5]. Choline-dependent potentiation of the cytotoxic activity of killer T cells, increased production of macrophage migration inhibition factor (MIF), and stimulation of proliferation of T lymphocytes induced by the graft versus host reaction, have been described [6, 7]. However, the effect of cholinergic stimulation on spontaneous lymphocyte adhesion has not previously been studied.

The aim of this investigation was to study the effect of the neurotransmitter ACh and its synthetic analog carbachol (CCh) on spontaneous adhesion of human lymphocytes in vitro, using blockers of muscarinic (atropine) and nicotinic (hexamethonium) ACh receptors.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes were isolated from healthy donors and their spontaneous adhesion studied by the method developed by the writer previously to study inhibition of lymphocyte adhesion without the addition of specific antigens [2, 3].

The wells of 96-well plastic plates (No. 3040, Falcon, USA) contained 0.1 ml of healthy human lymphocyte suspension ($2 \cdot 10^6$ cells/ml), 0.05 ml of medium 199 with 20% embryonic calf serum (ECS, from 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 a solution 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. The plates were then accurately turned over and incubated in the horizontal inverted position for another 30 min [1]. When the plates were

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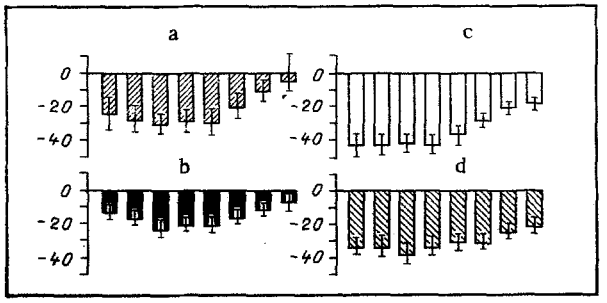

Fig. 1. Effect of ACh (a, b) and CCh (c, d) on spontaneous lymphocyte adhesion in serum-free medium (a, c) and in medium with ECS (b, d). Abscissa, concentration of ACh and CCh (columns from left to right): 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M; ordinate (here and in Fig. 2), LAI index (in %, M ± m).

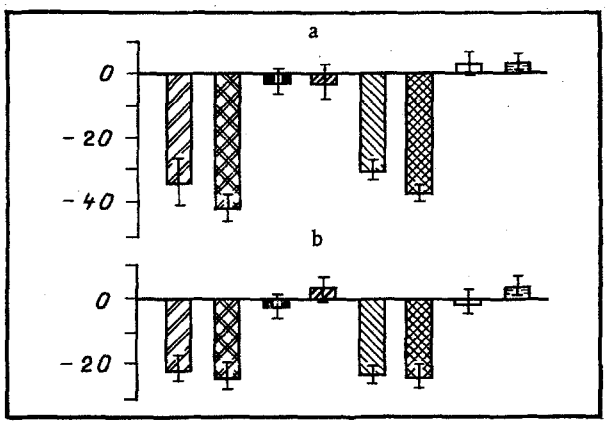

Fig. 2. Pharmacological specificity of stimulation of spontaneous lymphocyte adhesion in serum-free medium (a) and in medium with ECS (b). Abscissa (columns from left to right): stimulation of spontaneous lymphocyte adhesion by ACh (10^{-7} M); stimulation of spontaneous lymphocyte adhesion by CCh (10^{-7} M); stimulation of adhesion by ACh abolished by atropine (10^{-6} M); stimulation of response by CCh abolished on the addition of atropine (10^{-6} M); no effect of hexamethonium (10^{-6} M) on stimulation of spontaneous lymphocyte adhesion by ACh; no effect of hexamethonium (10^{-6} M) on stimulation of spontaneous lymphocyte adhesion by CCh (10^{-7} M); no effect of atropine (10^{-6} M) on spontaneous lymphocyte adhesion; no effect of hexamethonium (10^{-6} M) on spontaneous lymphocyte adhesion.

turned over the contents of the wells were retained due to the forces of surface tension and did not spill out provided that the edges of the wells were absolutely clean and dry. In this way nonadherent lymphocytes were separated from those which adhered to the bottom of the wells.

Nonadherent cells were transferred from the wells by means of an automatic micropipet into test tubes containing a 3% solution of acetic acid, and they were counted in a Goryaev chamber. The reaction was assessed by the usual formula for studying inhibition of lymphocyte adhesion, by means of a lymphocyte adhesion inhibition index (LAI index):

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

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

EXPERIMENTAL RESULTS

Investigation of the effect of ACh on spontaneous adhesion of healthy human lymphocytes showed (Fig. 1) that ACh potentiates lymphocyte adhesion, and that the optimal concentration range of ACh for this stimulation was 10^{-8}-10^{-6} M both in medium without serum and in medium with ECS. Similar results were obtained with CCh, whose activity remained at a significant level down to the minimal concentration investigated (10^{-10} M; Fig. 1). Unlike the results obtained with ACh, the presence of serum in the medium had virtually no effect on stimulation by CCh, indicating that the activating effect of ACh in medium with ECS may be reduced due to decomposition of the transmitter by serum enzymes.

To study the pharmacological specificity of the stimulation of spontaneous lymphocyte adhesion by cholinergic stimulation, the effect of addition of specific blockers of acetylcholine receptors — atropine and hexamethonium — to the culture medium was investigated. It will be clear from Fig. 2 that the addition of atropine completely blocked the stimulating effect of both ACh and CCh, both in serum-free medium and in medium with ECS, whereas hexamethonium had no effect on lymphocyte adhesion. Incidentally, the addition of both cholinolytics to the culture medium with or without ESC did not affect the adhesive properties of the lymphocytes.

Cholinergic stimulation of spontaneous lymphocyte adhesion is thus mediated by muscarinic ACh receptors.

The study of the effect of cholinergic drugs on lymphocyte adhesion is also of practical importance, for the method of spontaneous lymphocyte adhesion is used as a test of functional activity of human lymphocytes and it lies at the basis of the lymphocyte adhesion inhibition test, which is used clinically for diagnostic and prognostic purposes, and also to evaluate specific T-cell immunity, in patients with cancer in particular [3, 8].

The mechanism of this phenomenon is evidently based on elevation of the level of cGMP, which has a stimulating action on several immunologic responses [6]. Elevation of the cGMP level by cholinergic stimulation has been demonstrated in brain, heart, lung, and uterine tissues and in lymphocytes; in these last cells a threefold increase in the intracellular cGMP concentration was observed only 10 min after stimulation with ACh in a concentration of 10^{-6} M [6, 7]. It has been suggested [6] that the action of cholinergic agents on the corresponding receptor structures on the cell surface activates membrane-bound guanylate cyclase which, in turn, stimulates cGMP formation from the specific substrate (GTP).

The present investigation thus demonstrated, by the use of the spontaneous lymphocyte adhesion test, that ACh and CCh potentiate the adhesive properties of human lymphocytes in vitro. This potentiation was completely abolished by the specific muscarinic ACh receptor blocker atropine, but not by hexamethonium, which blocks nicotinic ACh receptors. This indicates that potentiation of spontaneous adhesion of human lymphocytes by the cholinergic drugs tested is mediated through their effect on muscarinic ACh receptors.

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