other glandular tissues which have been studied is the relatively high frequency of discovery of a stable rate of protein synthesis. Whether this difference is due to pathological changes in the stomach tissue will be made clear by future research.

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ACTION OF KETOTIFEN ON MITOGEN-INDUCED PROLIFERATIVE RESPONSE OF HUMAN MONONUCLEAR CELLS

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The present writers [1, 2] and other investigators [8, 10, 12] have shown that one point of application of the action of the antiallergic agent ketotifen, which gives multiple pharmacological effects [7], is the target cells of allergy, namely mast cells and basophils. The stabilizing action of ketotifen on the target cells of allergy is mediated through its membranotropic action [2], possibly by blocking membrane calcium channels [8]. The above remarks suggest that the action of ketotifen may also extend to other cell systems involved in the allergic response and, in particular, to immunocompetent cells, with which certain particular features of the therapeutic action of the drug may be linked. Accordingly the aim of the present investigation was to study the action of ketotifen on the mitogen-induced proliferative response of human peripheral blood lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) were isolated from heparinized blood from clinically healthy persons (six men aged 25-35 years) by centrifugation on a Ficoll-Verografin density gradient (density 1.080 g/cm^3). The isolated MNC were suspended in medium No. 199 containing 10% inactivated embryonic calf serum, HEPES (5 mM), glutamine (20 mM), and monomycin (100 U/ml) and cultured in flat-bottomed 96-well plates (Falcon Plastics, USA). The mitogens used were phytohemagglutinin (PHA; from Difco), concanavalin A (con A; from Sigma, USA), and pokeweed mitogen (PM; Sigma, USA). In the case of preliminary treatment of the MNC with ketotifen (Sandos, Switzerland) the cells (2×10^6) were preincubated in the presence of the drug in 2 ml of medium in glass flasks with bottom area of 4.1 cm^2. The viability of the cells (detected by uptake of trypan blue) cultured in the presence of the concentrations of ketotifen used was indistinguishable from that in the corresponding control. The proliferative response of MNC was estimated by the method described previously [3] based on incorporation of [^{3}H]thymidine, added in a dose of 1 μCi per well 6 h before the end of 72 h of culture. Protein

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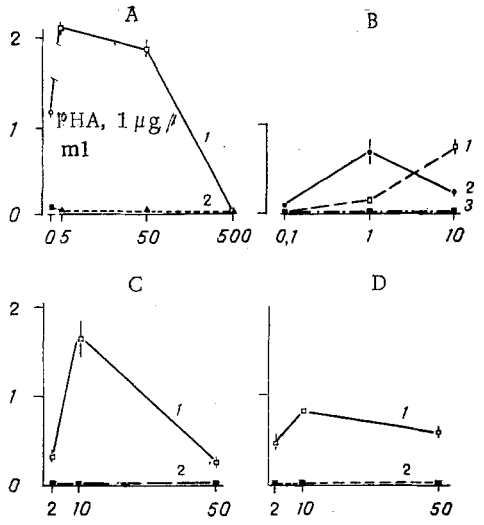

Fig. 1. Action of ketotifen on proliferative response of MNC induced by PHA (A, B), con A (C), and PW (D). A, C, D: Cells incubated with or without ketotifen and mitogens from beginning of culture for 72 h. A: 1) In the presence of PHA in concentration of 1 μg/ml; 2) without PHA; B: cells preincubated for 4 h without (1) or with ketotifen in a concentration of 250 μM (2), then washed twice, treated with PHA, and incubation continued until 72 h, or cells were incubated with PHA and ketotifen (250 μM) for 72 h (3); C and D: 1) without ketotifen; 2) with ketotifen (500 μM). Abscissa, concentration of ketotifen (A, in μM), of PHA (B, in μg/ml), con A (C, in μg/ml), and PW (D, in μg/ml). A and B) logarithmic scale; ordinate, incorporation of [^{3}H]thymidine (in cpm·10^{-4}).

synthesis was assessed by the method described in [5] based on incorporation of [^{14}C]-L-leucine (specific activity 37 mCi/mmole), added in a dose of 1 μCi per well 24 h before the end of culture of MNC for 48 h, into the cells. At the end of culture the cells were transferred to fiberglass filters, using a cell sampler, and radioactivity was counted in a Mark III liquid scintillation counter. The reaction was read as the number of counts per minute. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Ketotifen, within the range of concentrations tested (from 5 to 500 μM) caused no appreciable proliferative response, but depending on its concentration, it inhibited spontaneous proliferation of MNC (Fig. 1A, 2; Fig. 2B). Ketotifen, present in the medium throughout the period of incubation of the cells, had a different action on the proliferative response induced by PHA in a dose of 1 μg/ml. In low concentrations (5 and 50 μM) ketotifen potentiated, but in a higher concentration (500 μM) it completely blocked the response induced by PHA (Fig. 1A). In a concentration blocking the proliferative response of MNC to PHA, ketotifen also blocked cell proliferation induced by other mitogens (con A and PM) in all concentrations tested (Fig. 1C, D). These experiments determined the choice of concentrations of ketotifen for the subsequent tests. If the cells were treated beforehand with ketotifen (250 μM) for 4 h, then washed and tested with PHA, the blocking action of a high concentration of ketotifen was not exhibited (Fig. 1B). The character of the curves in Fig. 1 clearly corresponds to only a certain degree of inhibition of the cell response. These experiments thus indicate that interaction between ketotifen and the cells is reversible, confirming data obtained previously on other cell systems [2].

The action of ketotifen on the proliferative response of MNC induced by PHA is illustrated in Fig. 2A. As is evident (Fig. 2A, 1) the dose–response curve consists of two components, corresponding to testing of PHA in doses of 0.03 to 0.3 and from 0.3 to 10 μg/ml, with a maximum response at doses of PHA of 1-3 μg/ml.

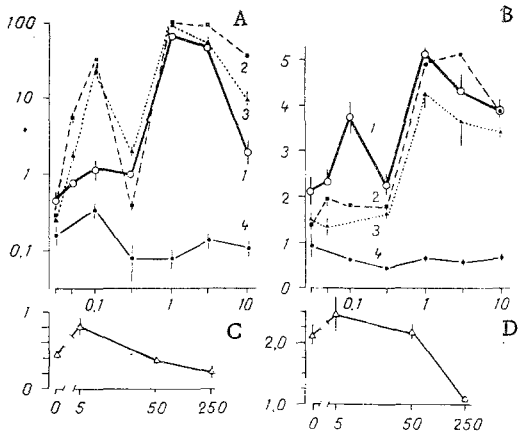

Fig. 2. Action of ketotifen on proliferation (A, B) of MNC and protein synthesis (C, D) in MNC without (B, D) and in the presence (A, C) of PHA. 1) Without ketotifen; 2) with ketotifen (5 μM); 3) with ketotifen (50 μM); 4) with ketotifen (250 μM); abscissa (logarithmic scale), PHA concentration (A and C, in μg/ml) and ketotifen (B and D, in μM); ordinate, incorporation (in cpm·10^{-3}) of [^{3}H]thymidine (A and B) and [^{14}C]-L-leucine (C and D). A) Logarithmic scale.

In the lowest concentration tested ketotifen (5 μM) induced an almost 30-fold increase in amplitude of the peak of the first component and a significant increase in the second component of response of the cells to PHA (Fig. 2A, 2). An increase in the ketotifen concentration (up to 50 μM) was accompanied by some smoothing of its action (Fig. 2A, 3), whereas testing ketotifen in an even higher concentration (250 μM) blocked the proliferative response of MNC to PHA (Fig. 2A, 4). Under the conditions used, incidentally, the dose–response curve for PHA was not shifted toward different concentrations of mitogen. This last fact confirmed that the action of low concentrations of ketotifen illustrated in Fig. 1A truly reflects its potentiating effect on PHA-induced proliferation of MNC. The curve obtained on testing a blocking concentration of ketotifen (Fig. 2A, 4), although steady, nevertheless repeats the character of the control dose–response curve for PHA (Fig. 2A, 1). This fact, and also data described above, suggest that ketotifen in a blocking concentration does not disturb the stages of interaction of mitogen with the cells or, correspondingly, the phase of induction of the proliferative response. If this is so, the presence of ketotifen in the early stage of activation of the cells ought not to permit its blocking action. In fact, during incubation of the cells in the presence of PHA and ketotifen (250 μM) during a sufficiently long period (4 h) for activation of MNC, followed by washing twice, the blocking action of ketotifen was not exhibited and the proliferative response was only a little below that in the corresponding control (in the absence of ketotifen) — Fig. 3A. The blocking action of a high concentration of ketotifen (500 μM) on proliferation induced by all three doses of PHA tested was manifested on the addition of ketotifen to the cells from 0 to 36 h of culture (Fig. 3B, 3, C, 3, D, 3), and the cells emerged from the inhibitory action of the drug only after 36 h of culture (toward 54 h). Since accumulation of most cells in the S phase took place after 48 h of culture of the lymphocytes with PHA [4], the character of the kinetics of the blocking action of ketotifen may be evidence that the drug tested acts on stages before the S phase. The proliferative response, potentiated by low concentrations of ketotifen, also had a tendency to return to the original level at the same time intervals.

The action of ketotifen, which can block H_1-receptors [7], on the proliferative response of MNC is evidently not connected with this property, for H_1-antihistamine preparations, at least in noncytotoxic concentrations, have no action similar to ketotifen on MNC proliferation [6].

Since ketotifen acts, not on the phase of induction, but on cell proliferation as such, testing the action of ketotifen on mitogen-induced potentiation of protein synthesis, required for cell proliferation, was justified [9, 15]. Tests showed that the increase in protein synthesis induced by PHA (Fig. 2C, 1), like the proliferative response, consists of two components.

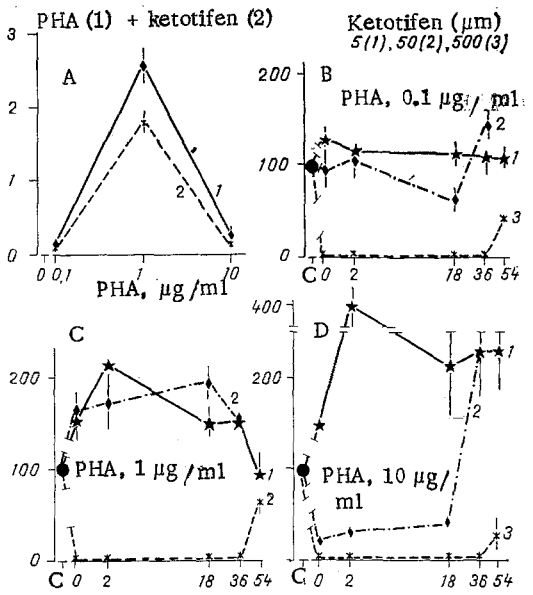

Fig. 3. Dependence of action of ketotifen on proliferative response of MNC, induced by PHA, on duration of incubation of cells in presence of ketotifen. A) Cells preincubated with PHA without (1) or with ketotifen in concentration of 250 μM (2) for 4 h, then washed twice, and incubation continued until 72 h; B-D) cells incubated with PHA in concentration of 0.1 μg/ml (B), 1 μg/ml (C), or 10 μg/ml (D), after which, at the specified time interval, ketotifen was added to them in concentrations of 5 μM (1), 50 μM (2), or 500 μM (3), and incubation continued until 72 h. Abscissa, PHA concentration (A, in μg/ml) and time of addition of ketotifen (B, C, and D, in h); ordinate: A) incorporation of [^{3}H]thymidine (in cpm × 10^{-4}), B, C, D) incorporation of [^{3}H]thymidine in absence of ketotifen (in percent of control).

A marked increase in protein synthesis corresponded in this case to the smoothed component of the proliferative response (compare Fig. 2A, 1 with Fig. 2C, 1) in the time interval used for recording. The possibility cannot be ruled out that the delay of proliferation of cells sensitive to low doses of mitogen is under the control of cell populations or processes responsible for the first component of the increase in protein synthesis. As will be shown below, small doses of ketotifen (5 and 50 μM), causing a sharp increase in the proliferative response of the cells (Fig. 2A, 2,3) completely abolished the first component of the increase in protein synthesis (Fig. 2B, 2,3).

Depending on dose, ketotifen inhibited the spontaneous level of protein synthesis (Fig. 2D) and the increase in protein synthesis induced by PHA, within the time interval used for recording (Fig. 2C, 2,3,4). The inhibitory action of low concentrations of ketotifen, as can be seen, had a greater effect on the first component of the increase in protein synthesis (Fig. 3C, 2,3).

It has been shown that under certain conditions inhibition of protein synthesis leads to increased production and secretion of mitogenic proteins by macrophages [14], a phenomenon known as "superinduction" of the cell function by inhibitors of protein synthesis. The superinduction phenomenon has also been described for glucocorticoid-induced secretion of tyrosine aminotransferase by liver cells [13] and interferon production [11]. The facts stated above may confirm the existence of a connection between the inhibitory action of low concentrations of ketotifen on protein synthesis within a certain time interval, and potentiation of the proliferative response of MNC. The particular features of the experimental procedure do not provide a basis for analysis of the connection between the action of ketotifen on the proliferative response of MNC and interference with protein synthesis at individual stages of stimulation of the cells by mitogen, which would be possible only in special investigations of

the kinetics of action of the drug on the two processes simultaneously, and comparison with the effect of inhibitors of protein synthesis with reversible and irreversible action.

Ketotifen has a therapeutic action only if used for a long time (about 3 months) [7]. When its effect on the systems of the body is evaluated, the action of ketotifen on lymphoid cells, manifested *in vitro* in the presence of extremely low concentrations of the drug, must therefore be taken into account. A special study of the possibility of realization of the immunotropic action of ketotifen *in vivo*, with, consequently, a more precise estimation of its effect on the allergic process, is also interesting.

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