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ACTION OF KETOTIFEN ON SELECTIVE AND UNSELECTIVE HISTAMINE LIBERATION FROM HUMAN BASOPHILS

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The antiallergic drug ketotifen possesses a multiple pharmacologic action, which includes H_1-antihistamine activity [7, 10]. The writers showed previously that ketotifen selectively (noncytotoxically) releases histamine from mast cells [2], unlike other H_1-antihistamine agents, which have an unselective (cytotoxic) histamine-releasing action (HRA) on the target cells of allergy [3]. In doses possessing HRA, ketotifen inhibits histamine secretion from mast cells due to another selective activator of histamine secretion [6]. There is reason to suppose that differences exist in the mode of action of ketotifen on mast cells and basophils, for the inhibitory action of ketotifen on histamine secretion from basophils is exhibited in the absence of its HRA [15]. Recently published data showing that ketotifen has a cytotoxic HRA on basophils are in conflict with the information described above [11].

To test these contradictions an investigation was carried out to assess the action of ketotifen on HRA and to inhibit histamine release from human basophils caused by selective and unselective liberators.

EXPERIMENTAL METHOD

Blood was obtained from six clinically healthy donors (two men and four women) aged from 20 to 40 years. Tests were carried out twice on cells from each donor, from whom blood was taken (up to 40 ml) at intervals of between 3 and 7 days. Mononuclear cells, enriched with basophils (2-4% of basophils) were separated from the blood by centrifugation on a one-step Ficoll-Verografin density gradient (1.080 g/cm^3) [13]. The cells were incubated without and in the presence of the test substances for the necessary period of time at 37°C in 200 or 400 µl of buffer of the following composition (in mM): Tris-HCl (from Sigma) 25, pH 7.6; NaCl 120, KCl 5, $MgCl_2$ 1.15, $CaCl_2$ 0.6, glucose 5; human serum albumin 0.3 mg/ml. A buffer of the same composition but without Ca^{++} and Mg^{++} was used to isolate and wash the cells. The reaction was stopped by addition of 2 ml of cold buffer to the cells and placing the tubes on ice. Cells were sedimented by centrifugation at 2000g and 4°C for 10 min. The supernatant was discarded and the histamine content determined microspectrofluorometrically in the residual portions without preliminary extraction [13]. Ketotifen hydrogen fu-

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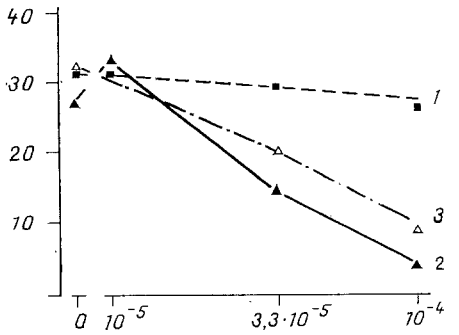

Fig. 1. Action of papaverine and iodoacetate on histamine release induced by con A. 1) In presence of glucose (5 mM); 2) in absence of glucose; 3) in presence of glucose and iodoacetate (13 μM). Cells were preincubated with the test substances for 30 min, after which con A (10 μg/ml) was added and incubation was continued for 30 min. Abscissa, papaverine concentration (in M); ordinate, histamine release (in %).

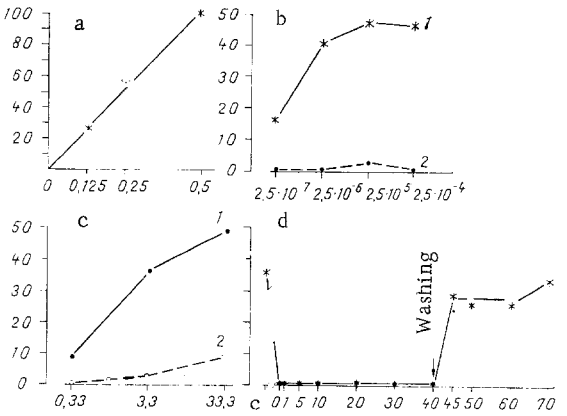

Fig. 2. Action of ketotifen on histamine release induced by anti-IgE-antibodies (a, b, d) and con A (c). 1) Control; 2) in presence of ketotifen. Cells preincubated with ketotifen (0.5 mM for 40 min (a-c) or time stipulated (d), after which the histamine secretion activator was added and incubation continued for 30 min. Arrow indicates time of washing cells free from ketotifen (d). Concentration of anti-IgE-antiserum $0.6 \cdot 10^{-5}$ (a) and $1.25 \cdot 10^{-6}$ liter/liter (d). Abscissa, ketotifen concentration (in mM, a); of anti-IgE-antiserum (in liters/liter, b); of con A (in μg/ml, c); time (in min, d). Ordinate, inhibition of histamine release (in %, a); histamine release (in %, b-d).

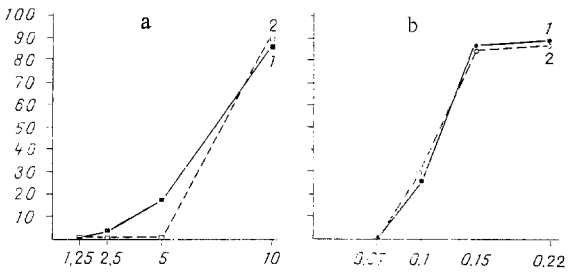

Fig. 3. Action of ketotifen on histamine release induced by mellitin (a) and chlorpromazine (b). 1) Control; 2) in presence of ketotifen (0.5 mM). Cells preincubated without and with ketotifen (40 min), after which the histamine liberator was added and incubation continued for 10 min. Abscissa, concentration of mellitin (in μg/ml, a) and chlorpromazine (in mM, b). Ordinate, histamine liberation (in %).

marate (Sandoz) was dissolved in distilled water immediately before use. The final concentrations of ketotifen did not disturb the histamine assay reaction.

Materials: Ficoll was from Pharmacia Fine Chemicals, Verografin from Spofa, orthophthalic aldehyde from Koch-Light Laboratories, sheep anti-IgE-serum with an antibody content of 0.2-0.5 mg/ml was from Pharmacia Diagnostic, sodium iodoacetate and concanavalin A (con A) were from Sigma, and papaverine hydrochloride was of Soviet origin.

EXPERIMENTAL RESULTS

The results showed that ketotifen in concentrations between 0.125 and 1.0 mM did not release histamine in any test on basophils from six individuals, in agreement with existing data [15]; they did not confirm the cytotoxic HRA of the drug on human basophils [11]. The only, although unlikely, explanation of the difference between these results may lie in differences between the cell donors, for the character of their selection is not mentioned in the paper cited [11].

The next step was to study the action of ketotifen on histamine secretion from basophils. Anti-IgE-antibodies and con A were chosen as activators of secretion. There is reason to suppose that con A, like anti-IgE-antibodes, is a selective activator of histamine secretion [14]. Moreover, the possibility cannot be ruled out that its HRA is similar to the action of anti-IgE-antibodies also in the respect that con A triggers histamine secretion through its interaction with IgE molecules fixed on basophils [9, 14].

To confirm that the histamine release induced by con A is noncytotoxic in character (dependent on energy consumption) the process was subjected to further tests. It will be clear from Fig. 1 that papaverine, under conditions (in the absence of glucose) in which, as the writers showed previously [8], ATP reserves in the cells are exhausted by blocking of cellular respiration, inhibits, depending on dose, histamine release induced by con A. Glucose, by restoring the ATP reserves, abolishes the inhibitory action of papaverine. Finally iodoacetate, in a concentration (0.013 mM) blocking the glycolytic pathway of ATP accumulation [12], in the presence of papaverine and glucose inhibits con A-induced histamine release from basophils, which confirms that it is an energy-dependent process and explains the use of con A as selective activator of secretion.

Fig. 2a illustrates the inhibition, dependence on the dose of ketotifen, of histamine release induced by anti-IgE-antibodies. Complete inhibition was achieved with ketotifen in a concentration of 0.5 mM. In this effective concentration ketotifen inhibits HRA of increasing concentrations of anti-IgE-antibodies (Fig. 2b) and con A (Fig. 2c) practically completely. The time course of the inhibitory action of ketotifen on histamine secretion induced by anti-IgG-antibodies corresponds to the rapid development of inhibition (Fig. 2d), maintained throughout the period of testing (up to 40 min) in the presence of ketotifen. Washing the cells to remove ketotifen was accompanied by rapid (at least in the course of the first 5 min) restoration of sensitivity of the cells to HRA of anti-IgE-antibodies (Fig.

2d). The rapid development of the inhibitory action of ketotifen is evidence at least that this effect is not linked with blocking of the energy-dependent stage of histamine secretion, due to ATP [8]. This is also confirmed by the fact that inhibition of histamine secretion by ketotifen occurs in both the absence and the presence of glucose.

The writers showed previously that H_1-antihistamine drugs inhibit histamine secretion from the target cells of allergy in doses in excess of those inducing cytotoxic histamine release, and this inhibitory action is linked with the cytotoxic property of the drugs [4]. A distinguishing feature of cytotoxic inhibitors of histamine secretion is that, in doses inhibiting secretion of the mediator they potentiate the HRA of other cytotoxic agents [4].

To test the possible linking of the inhibitory activity of ketotifen with its cytotoxic activity not manifested as HRA within the limits of the concentrations tested, the action of ketotifen was studied on histamine release induced by two different agents (mellitin and chlorpromazine), whose cytotoxic HRA was demonstrated previously [4, 5].

In a dose inducing maximal inhibition of histamine secretion from basophils, ketotifen at least did not potentiate the cytotoxic HRA of mellitin (Fig. 3a) and chlorpromazine (Fig. 3b). The absence of correlation between inhibition of histamine secretion by ketotifen and its cytotoxic effect is also demonstrated by the reversibility of the inhibitory action of ketotifen after rinsing out from the cells (Fig. 2d).

Selective HRA of a given agent presupposes its possession of cell receptors. The absence of HRA of ketotifen on basophils and the manifestation of its selective HRA on mast cells [2] are thus evidence that only mast cells have receptors for ketotifen, through which secretion of the mediator is effected. It is not clear, however, whether this property is a specific feature of rat cells or whether it is also characteristic of human mast cells. Unfortunately the experimental testing of this hypothesis is difficult at present because of the impossibility of obtaining human mast cells for such tests.

This paper gives evidence that inhibition of histamine secretion from basophils by ketotifen is not linked with any possible cytotoxic action of the drug. The rapid development of inhibition and its rapid disappearance after washing the cells shows that this effect is most probably mediated through surface receptors, and that penetration of ketotifen inside the cells is unnecessary for it to take place. The fact that the selective HRA of ketotifen is exhibited only on mast cells [2], and that its inhibitory action is expressed both on basophils ([15], present communication) and on mast cells [6] suggests that these two methods of action are realized through different receptor formations. This explanation admits the possibility of creating drugs with an inhibitory action like that of ketotifen, but without any HRA. This is a particularly interesting suggestion in view of the following circumstances. The writers previously put forward a theory of noncytotoxic involvement of target cells (mast cells and basophils) in the allergic reaction, and verified it experimentally [1]. This theory was the basis for a new approach to the pharmacologic control of allergic reactions, through inhibition of target cell function [1], which does not, however, eliminate the desirability of using antagonists of the mediators of allergy. It is natural to suggest that the optimal version of an antiallergic drug would be one which combines ability to inhibit the function of target cells, thus preventing secretion of mediators of allergy from them, and ability to block receptors of histamine — the most important mediator of allergy. To establish the validity of this approach to allergy control, the writers have studied several different antihistamine drugs, including some original Soviet preparations, for their ability to inhibit secretion of mediators of allergy from target cells [4]. Several H_1-antihistamine drugs have been shown actually to exhibit the properties of inhibiting histamine secretion from target cells induced by antigen or by other selective secretion activators [4]. However, determination of the mechanism of this action of the antihistamine agents shows that it is coupled with their cytotoxic HRA [3, 4] and it cannot therefore be used for clinical allergy control [4]. Ketotifen is an exception in this respect, for the inhibition of histamine secretion induced by it is not coupled with a cytotoxic effect. Accordingly ketotifen, which combines in itself the properties of an H_1-antihistamine drug and ability to inhibit, by a noncytotoxic mechanism, the secretion of mediators of allergy from basophils, is an example on which the principle of control of the allergic reaction enunciated above is based. However, not all properties of ketotifen satisfy demands which may be made on drugs with this type of action. First, ketotifen itself possesses selective HRA on mast cells [2] and, second, its inhibitory effect on histamine secretion is rapidly reversible. In our view the optimal solution would be selection of compounds combining the properties of

antagonists of allergy mediators with ability to inhibit their secretion in the absence of HRA, and provided that the inhibitory effect of the compound is preserved after its removal from the extracellular medium.

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INHIBITORY ACTION OF ANTIARRHYTHMIC PHENOTHIAZINE DRUGS ETHMOZINE AND ITS DIETHYLAMINO ANALOG ON PLATELET AGGREGATION AND METABOLISM OF ENDOGENOUS ARACHIDONIC ACID

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The new phenothiazine derivatives ethmozine and its diethylamino analog (DAAE) are the first compounds of the phenothiazine series which have marked antiarrhythmic activity under both experimental [1, 2] and clinical conditions [1, 3]. The psychotropic phenothiazines, namely trifluoperazine and chlorpromazine, are known to inhibit platelet aggregation [7, 14] and the formation of proaggregant metabolites of arachidonic acid (AA) [13]. These effects of the phenothiazine are due to blocking of activity of the Ca^{++}-binding regulatory protein, calmodulin [8, 12].

In the investigation described below, to obtain further information on the spectrum of action of cardiotropic phenothiazines, the effect of ethmozine and DAAE on aggregation of platelets and on the formation of AA metabolites in them was studied.

EXPERIMENTAL METHOD

To obtain platelet-rich plasma, blood from the cubital vein of healthy blood donors was collected into 3.8% sodium citrate (ratio of anticoagulants to blood 1:9) and centrifuged at

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