KETOTIFEN, A SELECTIVE HISTAMINE RELEASER

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Liberation of histamine from isolated rat mast cells induced by Ketotifen was shown to be inhibited when the ATP reserves in the mast cells are exhausted. Histamine release induced by Ketotifen is a temperature-dependent process and is blocked at a low temperature. It is concluded that Ketotifen is a selective liberator of histamine. The time course of histamine liberation induced by Ketotifen corresponds to partial return of the liberated histamine into the cell.

KEY WORDS: mast cells; histamine release; Ketotifen.

Ketotifen, with an antiallergic action similar to that of the disodium salt of cromoglycate (Intal), possesses antihistamine properties, comparatively low antiphosphodiesterase activity, and ability to inhibit the entry of Ca ions into mast cells under the influence of substance 48/80 [6, 7]. Comparison of the antiallergic action of Ketotifen, other antihistamines, and Intal shows that these properties cannot explain the special character of the antiallergic effect of Ketotifen observed clinically [8, 10]. Ketotifen also has a histamine-releasing action, the mechanism of which has not been established. Together with the others listed above, this property can be used to study the antiallergic action of Ketotifen.

In the investigation described below the mechanism of the histamine-releasing action of Ketotifen was studied.

METHODS

Male albino rats weighing 300-350 g were used. The method of isolating the mast cells, the design of the experiments, the composition of the solutions used, and the method of spectrofluorometric determination of histamine were described previously [1, 5]. Histamine liberation was expressed as percentages of its total content in a sample of mast cells. Ketotifen (4,9-dihydro-4-(1-methyl-4-piperidinylidene)-10H-benzo[4,5]cyclohepta[1,2-b]thiophene-10-OH fumarate; mol. wt. 425.5) was obtained from Sandoz Ltd. (Switzerland).

RESULTS AND DISCUSSION

Papaverine, in a dose (0.1 mM) which, as the writers showed previously [5], leads to exhaustion of ATP reserves in the mast cells, inhibited the histamine-releasing action of Ketotifen, but addition of glucose (10 mM), which restores the ATP content in the cells through glycolytic accumulation of ATP [5], to the medium reduced the inhibitory action of papaverine (Fig. 1A).

Simultaneous addition of papaverine (0.05 mM) and iodoacetate, as an inhibitor of the glycolytic pathway of ATP accumulation [9], in the presence of glucose (10 mM) led to inhibition, dependent on the dose of iodoacetate, of Ketotifen-induced histamine release (Fig. 1B).

The inhibitory action of papaverine was not connected with its antiphosphodiesterase activity or with the corresponding increase in the concentration of cyclic AMP in the cells, for under the experimental conditions used (in the absence of glucose) papaverine caused no increase in the cyclic AMP concentration in the mast cells [3, 5] on account of exhaustion of the ATP reserves. The antiphosphodiesterase compound 1-methyl-3-isobutylxanthine (MIBX), in concentrations (up to 0.8 mM) in which it caused a 20-fold increase in the cyclic AMP concentration in mast cells [5], caused only slight inhibition of Ketotifen-induced histamine release, which was not potentiated by iodoacetate (Fig. 1C). As Fig. 1C shows, in this case only the combined effect of the inhibitory action of MIBX and iodoacetate (0.013 mM) was observed.

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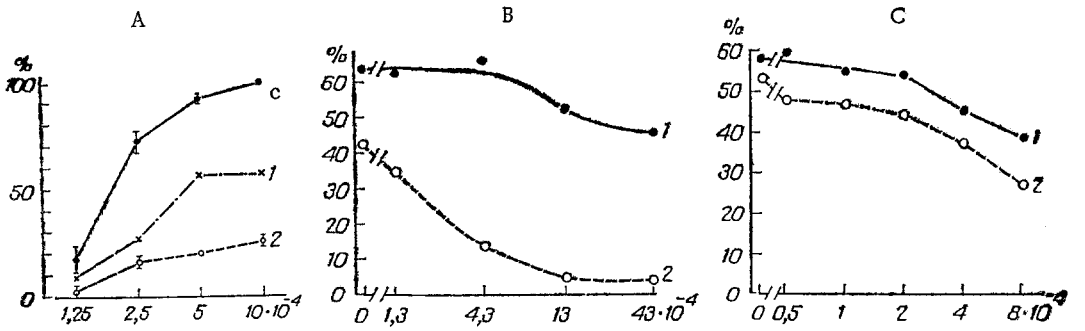

Fig. 1. Inhibition of histamine release induced by Ketotifen. A) Action of papaverine (0.1 mM) in the presence (1) and absence (2) of glucose (10 mM). c) Control; B) action of iodoacetate together with glucose (10 mM) in the absence (1) and presence (2) of papaverine (0.05 mM). C) Action of MIBX in the absence (1) and presence (2) of iodoacetate (0.013 mM). Cells preincubated at 38°C for 10 min in 200 µl buffer solution in the absence or presence of inhibitors. Ketotifen was then added and incubation continued for 5 min. The reaction was stopped by transfer of 20 µl of the cell suspension into 2 ml of cold (4°C) buffer. Spontaneous release of histamine 3.3 ± 0.8% (A), 3.3% (B), and 6.0% (C). Maximal histamine release 43.5 ± 5.0% (A). Concentration of Ketotifen 0.5 mM (B and C). Ordinate, histamine release [in % of maximal (A) and in % (B and C)]; abscissa, concentration of Ketotifen (A), iodoacetate (B), and MIBX (C) (in M).

Histamine release induced by Ketotifen was completely inhibited at a low temperature (3°C) and increased sharply when the temperature during the incubation period was raised to 18 and 37°C (Fig. 2).

Determination of the dependence of Ketotifen-induced histamine release on time showed that the quantity of histamine released reached a maximum during the first 20 sec of incubation, after which it fell to reach a stable level at 2 min (Fig. 3). That this character of the curve was not due to interaction of the Ketotifen with the liberated histamine followed by its inactivation is shown by the curve of the absolute histamine content in the cells shown in the same Fig. 3, which is the mirror image of the histamine release curve. Clearly the histamine content fell sharply to begin with, after which it recovered a little in the cells.

Known histamine-releasing agents can be subdivided, depending on the mechanism of their action, into unselective (cytotoxic) and selective (noncytotoxic) liberators of histamine [4]. The latter are known to include a specific antigen (allergen). The main characteristic feature of selective liberators is active, energy-dependent histamine release caused by ATP [4]. The results of the present experiments show that if ATP reserves were exhausted in the mast cells, Ketotifen-induced histamine release was inhibited, and procedures restoring the ATP content abolished the inhibitory action of inhibitors of ATP accumulation. Like the action of other selective histamine liberators [4], the histamine-releasing action of Ketotifen was blocked at a low temperature, further evidence that this type of histamine release corresponds to an active process. The results described above thus show that Ketotifen is a histamine liberator of selective type.

Many antihistamine agents blocking H_1 receptors are known to be unselective histamine liberators [2]. In this respect Ketotifen, which also has antihistamine properties, is an exception to the rule. Its selective histamine-releasing activity is interesting also because of the following fact. If this activity is also manifested *in vitro*, prolonged use of Ketotifen leading to a definite liberation of the same mediators that are liberated during the allergic reaction ought, because of well-known physiological principles, to be accompanied by a decrease in the sensitivity of the tissue receptors to these mediators, and this could explain, although perhaps only partly, the antiallergic action of prolonged administration of Ketotifen. In this connection the study of the sensitivity of tissues to mediators of anaphylaxis during prolonged administration of Ketotifen would be interesting on its own account.

The time course of histamine liberation during the action of Ketotifen was shown to cor-

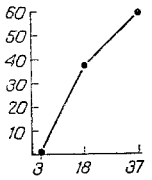

Fig. 2. Temperature dependence of histamine release induced by Ketotifen (0.1 mM). Duration of incubation 3 min, spontaneous histamine release 2.5%. Ordinate, histamine release (in %); abscissa, temperature (in °C)

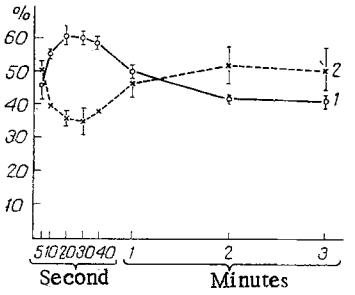

Fig. 3. Time course of histamine release induced by Ketotifen (1.0 mM). 1) Histamine release (in %); 2) histamine concentration in cells (in % of initial). Cells were incubated at 38°C in 200 µl buffer solution in the absence or presence of Ketotifen for period indicated along abscissa. Reaction stopped by addition of 2 ml cold buffer solution. Volume of cell suspension used to determine histamine 200 µl. Ordinate, histamine release (in %) and histamine content in cells (in % of initial). Spontaneous histamine release 4.6 ± 1.1%; abscissa, duration of incubation of cells in presence of Ketotifen.

respond to the reentry of some of the liberated histamine into the cells. Since other known histamine liberators do not possess this property it is unlikely that this well marked return of histamine into the cells can be explained entirely by increased permeability of the cell membrane during mediator secretion from the mast cells. One acceptable suggestion would be that Ketotifen itself enters the cell during the period of increased membrane permeability, and that it transports histamine in the manner of a carrier.

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