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INTERACTION OF HISTAMINE H_1- AND H_2-RECEPTOR ANTAGONISTS WITH HISTAMINE UPTAKE AND METABOLISM BY GUINEA-PIG ISOLATED ATRIUM AND MOUSE NEOPLASTIC MAST CELLS *in vitro*

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1 Burimamide, metiamide, chlorpheniramine, triprolidine and cocaine, were tested as inhibitors of histamine uptake and metabolism in the guinea-pig atrium and in mouse neoplastic mast cells.

2 Cocaine did not affect the uptake and metabolism of histamine, either in the atrium or in the mast cells. All the antihistamines tested blocked the uptake and metabolism of histamine in both preparations. The order of potency was burimamide > chlorpheniramine > triprolidine > metiamide in the atrium; and burimamide > metiamide > triprolidine > chlorpheniramine, in the mast cells.

3 Comparison of the present results with the antihistamine activity of these blocking agents suggests that no correlation exists between the receptor blocking activity and the ability of these substances to act as inhibitors of histamine uptake and metabolism.

Introduction

Traditional antihistamines (H_1-receptor blocking agents; Ash & Schild, 1966) as well as the more recent H_2-receptor blocking agents (burimamide and metiamide: Black, Duncan, Durant, Ganellin & Parsons, 1972; Black, Duncan, Emmet, Ganellin, Hesselbo, Parsons & Willie, 1973) are thought to inhibit the response to histamine by a primary action at the receptor site. However, it is possible that some antihistamines might antagonize the effects of histamine by preventing the uptake of both exogenous and locally released histamine. In fact, a variety of antihistamines potentiate the arterial pressor response and the positive chronotropic action of noradrenaline in the rat heart, by inhibiting, like cocaine, the amine uptake at the neuronal membrane (Isaac & Goth, 1965; Johnson & Kahn, 1966; Isaac & Goth, 1967).

The purpose of the present study was to determine whether H_1- and H_2-receptor antagonists prevent the uptake of histamine in guinea-pig heart and mouse neoplastic mast cells and if so, to see whether this effect is related to their histamine receptor blocking properties.

The experiments were carried out on guinea-pig atria and mouse neoplastic mast cells, in an attempt to differentiate between the effects of triprolidine, chlorpheniramine, burimamide and metiamide in preparations in which the accumulation of histamine is mainly due to uptake (mast cells: Moroni, Buiatti & Mannaioni, 1972), or to uptake and metabolism (isolated atrium: Mannaioni & Moroni, 1973).

Methods

Experiments on isolated atria

The interactions of antihistamines with histamine uptake and metabolism were studied in isolated left atria of guinea-pigs prepared according to the procedure described by Furchgott, Kirpekar, Rieker & Schwab (1963). Each atrium was placed under a tension of 1 g in a 5 ml muscle chamber and perfused with Tyrode solution at 30° C, through which a mixture of 97% O_2 and 3% CO_2 was bubbled. The composition of the perfusion fluid (mM) was: NaCl 136.9; KCl 2.7; $CaCl_2$ 1.8 (titrated); $MgCl_2$ 1.0; NaH_2PO_4 0.4; $NaHCO_3$ 11.9; glucose 5.6. The pH of the solution was 7.45. The preparations were stimulated electrically (0.5 ms duration; twice threshold voltage; 2 Hz) and contractions were recorded with a Battaglia-Rangoni polygraph. After an equilibration period,

the atria were perfused either with [^{14}C]-histamine or with [^{14}C]-histamine plus various concentrations of drugs over a period of 30 min, and then washed for 10 min to remove extracellular histamine (Mannaioni & Moroni, 1973).

The chronotropic response to noradrenaline in the presence of cocaine and antihistamines was measured on isolated, spontaneously beating atria of the guinea-pig. The atrial beats were recorded by attaching the atria to an isometric Grass transducer under a tension of 1 g, connected to a Battaglia-Rangoni polygraph.

Having established the resting atrial rate, (±)-noradrenaline was added to the bath at 2 min intervals in final concentrations ranging from 10^{-10} to 10^{-5} M. After this procedure, the atrium was repeatedly washed until the control rate was re-established. One of the test compounds was then added to the bath, followed by the cumulative graded concentrations of the amine.

Experiments on mast cells

The experiments were carried out on a clone of murine neoplastic mast cells originating from the Furth mastocytoma (HC subline: Mannaioni, Fischer & Giarman, 1968). The clone was maintained as an ascitic tumor in LAF_1 mice (Jackson Memorial Laboratory). The histamine content of the cells continuously grown in mice was fairly constant (Mannaioni, 1970). Periodic microscopic examination revealed a quite uniform cell population, accounted for almost completely by mast cells (95-98%).

Treatment with drugs was carried out as follows: cells from mice bearing the ascitic tumour were removed (usually 5-7 days after the inoculum) by aspiration of peritoneal fluid, collected by centrifugation and washed with 5 ml of ice-cold Tyrode solution. The cells were harvested and resuspended in a medium containing [^{14}C]-histamine, and [^{14}C]-histamine plus different concentrations of antihistamines. After incubation for 1 h (gas phase: air; pH 7.45) a cell count was taken; the cells were harvested, washed three times and extracted. All samples were run in duplicate. All the drugs were dissolved in Tyrode at pH 7.45 and added to the cell suspension in a ratio never exceeding 1 : 10 ml.

Extraction and analyses

Atria and mast cells were homogenized in 0.4 N perchloric acid and suitable aliquots were taken for the assay of total radioactivity, histamine and methylhistamine.

In the atria, total radioactivity was measured in the perchloric acid extract. The [^{14}C]-histamine assay was carried out according to the isotope dilution method of Schayer (1968). The [^{14}C]-methyl-histamine was assayed by the method of Snyder, Axelrod & Bauer (1964). Aliquots of the perchloric acid extracts of the atria were extracted into alkaline chloroform. After washing the chloroform layer, methyl-histamine was transferred to acid and counted. The difference between the total radioactivity and that referring to [^{14}C]-histamine plus [^{14}C]-methyl-histamine accounted for the sum of the other metabolites.

In mast cells, assay by the isotope dilution method showed that [^{14}C]-histamine accounted for all the radioactivity, so that in further experiments this procedure was considered no longer necessary, and the amount of radioactivity found in perchloric extracts was directly referred to as [^{14}C]-histamine.

The endogenous histamine content of the atria and mast cells was determined by means of a biological assay carried out on the guinea-pig ileum as described by Giotti, Guidotti, Mannaioni & Zilletti (1966).

Drugs

Histamine-[ring 2-^{14}C] dihydrochloride, specific activity 54 mCi/mmol, was purchased from the Radiochemical Centre, Amersham.

Burimamide and metiamide were obtained from Dr J.W. Black, Smith Kline & French Laboratories. Other substances used were: triprolidine hydrochloride, (Wellcome); chlorpheniramine maleate, (Schering); cocaine hydrochloride, (Carlo Erba); (±)-noradrenaline hydrochloride, (Calbiochem); histamine dihydrochloride, (Calbiochem).

Results

Chronotropic responses to noradrenaline in the presence of cocaine and antihistamines

Figure 1 shows that triprolidine did not significantly shift the dose-response curve to noradrenaline, while chlorpheniramine increased the positive chronotropic effect of noradrenaline, especially at the lowest concentrations. Higher concentrations of antihistamines directly depressed the atrial rate, while lower concentrations were ineffective.

Burimamide and metiamide failed to shift the dose-response curve to noradrenaline, even at high concentrations, while cocaine, at a concentration of 10^{-6} M shifts this curve upwards and to the left (Figure 2). None of the antihistamines studied modified the atrial rate at the concentrations used.

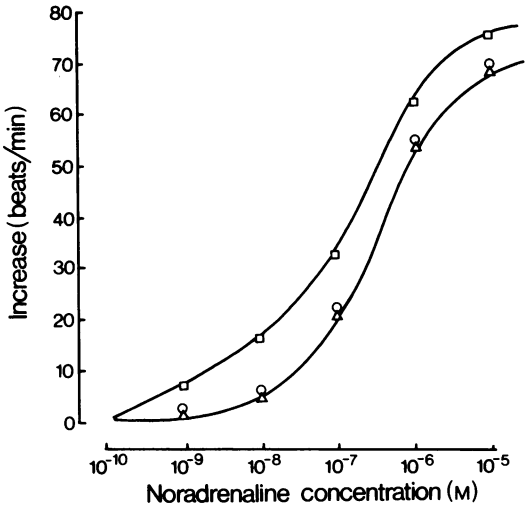

Figure 1 Effect of triprolidine and chlorpheniramine on the chronotropic action of noradrenaline in guinea-pig atria. Control (○); triprolidine 10^{-6} M (△); chlorpheniramine 10^{-6} M (□). The control curve is the mean of 10 experiments; the curves for triprolidine and chlorpheniramine are both the mean of 5 experiments. For each point the s.e. mean fell between 1.7 and 7.8 beats/minute.

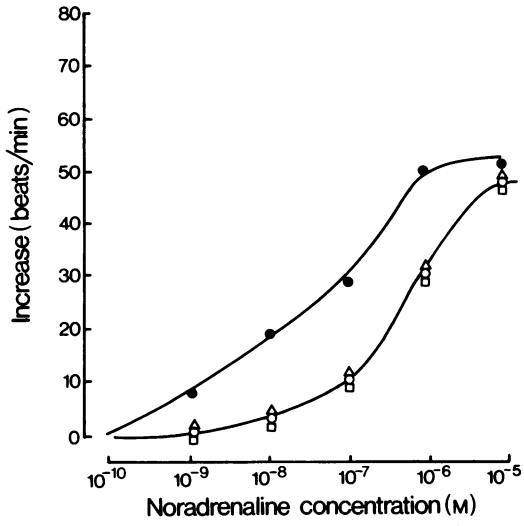

Figure 2 Effect of cocaine, burimamide and metiamide on the chronotropic action of noradrenaline in guinea-pig atria. Control (○); cocaine 10^{-6} M (●); burimamide 3×10^{-4} M (△); metiamide 10^{-4} M (□). The control curve is the mean of 15 experiments; the other curves are each the mean result of 5 experiments. For each point the s.e. mean fell between 1.3 and 7.1 beats/minute.

Effects of drugs on histamine uptake and metabolism in the atria

At the highest concentrations used, none of the drugs studied (triprolidine; chlorpheniramine; burimamide; cocaine) altered the endogenous histamine content.

The H_1-receptor blocking agents, triprolidine and chlorpheniramine, prevented the accumulation of total radioactivity, as well as the formation of methylhistamine, in a dose-dependent fashion. However, at the same concentrations they did not significantly block the uptake of the unchanged histamine (Table 1). By measuring the drug concentration required to produce a 50% inhibition of histamine methylation (IC_{50}), it was possible to show that chlorpheniramine was more effective than triprolidine in affecting this process (Table 3).

The H_2-receptor blocking agents, burimamide and metiamide, behave similarly to triprolidine and chlorpheniramine in inhibiting the accumulation of total radioactivity and the formation of methyl-histamine, without impairing the uptake of unchanged histamine. However, the IC_{50} clearly revealed that burimamide is the most active compound, while metiamide blocked the uptake of radioactivity and the histamine methylation only at high concentrations.

It is interesting to note that cocaine, at the concentration used (10^{-6} M) was devoid of any significant action on the accumulation and metabolism of histamine (Table 1).

Effect of drugs on histamine uptake by mast cells

Cell viability and endogenous histamine content were unchanged by exposure of cells to drug concentrations up to 10^{-4} M.

Cocaine was completely ineffective in blocking histamine uptake at all concentrations used. Chlorpheniramine showed a clear inhibition of the uptake process only at high concentrations. Triprolidine and metiamide were almost equiactive, although they blocked histamine uptake more effectively than chlorpheniramine. Burimamide was clearly the most active compound, showing a significant inhibition of histamine uptake even at low concentrations (Table 2).

The IC_{50} of the four antihistamines were, for burimamide 1.8 μM, for metiamide 28 μM, for triprolidine 20 μM and for chlorpheniramine 60 μM.

Table 1 Effect of antihistamines and cocaine on the uptake and metabolism of [^{14}C]-histamine by guinea-pig isolated atria

Treatment	*Concentration of drug* (M)	*Total radioactivity*	*Methyl-histamine* (*d/min x 10^{-3}*) *per g*	*Histamine*
Control	–	170 ± 7	43 ± 3	7.8 ± 0.7 (12)
Triprolidine	3.10^{-7}	152 ± 20	40 ± 2	7.5 ± 1 (4)
	10^{-6}	133 ± 18*	34 ± 5	6.1 ± 1.3 (4)
	10^{-5}	125 ± 4*	28 ± 4*	6.2 ± 1.2 (4)
	10^{-4}	72 ± 0.7*	7.9 ± 1*	6.2 ± 1.5 (4)
Chlorpheniramine	3.10^{-7}	180 ± 20	46 ± 3	7.6 ± 1 (4)
	10^{-6}	133 ± 2*	28 ± 3*	6.2 ± 0.9 (4)
	10^{-5}	123 ± 7*	17 ± 2*	5.2 ± 1.2 (4)
	10^{-4}	71 ± 6*	8 ± 0.9*	5.2 ± 1.7 (4)
Burimamide	3.10^{-7}	124 ± 7*	24 ± 3*	9.1 ± 2 (4)
	10^{-6}	71 ± 3*	14 ± 4*	8.7 ± 2 (4)
	10^{-5}	36 ± 5*	5 ± 0.5*	8.3 ± 15 (4)
	10^{-4}	26 ± 4*	5 ± 0.5*	9.6 ± 4 (4)
Metiamide	3.10^{-7}	166 ± 17	38 ± 2	8.1 ± 0.7 (4)
	10^{-6}	140 ± 9*	42 ± 3	8.1 ± 0.7 (4)
	10^{-5}	109 ± 7*	37 ± 2	8 ± 1.2 (4)
	10^{-4}	53 ± 7*	8 ± 0.9*	7.3 ± 3 (4)
Cocaine	10^{-6}	141 ± 7	47 ± 9	8 ± 2 (4)

Atria were incubated with [^{14}C]-histamine (100 ng/ml) for 30 min and washed for 10 min before extraction and analysis.
Number of experiments in parentheses. Values are means with s.e.
* Statistically significant difference from controls ($P < 0.01$) according to the analysis of variance.

Table 2 Effect of antihistamines and cocaine on the uptake of [^{14}C]-histamine by murine neoplastic mast cells

Concentration of drugs (M)	*Burimamide*	*Metiamide*	*Triprolidine*	*Chlorpheniramine*	*Cocaine*
		Histamine (d/min x 10^{-6}) cells			
0 (controls)	5308 ± 460 (4)	6087 ± 580 (4)	3121 ± 315 (4)	3705 ± 416 (4)	3608 ± 300 (4)
10^{-7}	–	6656 ± 800 (4)	–	3797 ± 633 (4)	– –
10^{-6}	–	6520 ± 1000 (4)	2633 ± 350 (4)*	4157 ± 589 (4)	–
3×10^{-6}	2047 ± 70 (4)*	6330 ± 980 (4)	–	3974 ± 564 (4)	3888 ± 50 (4)
10^{-5}	1066 ± 63 (4)*	4600 ± 648 (4)*	2043 ± 137 (4)*	3853 ± 662 (4)	4188 ± 350 (4)
3×10^{-5}	494 ± 26 (4)*	2891 ± 200 (4)*	935 ± 170 (4)*	3439 ± 873 (4)	4216 ± 425 (4)
10^{-4}	215 ± 14 (4)*	1255 ± 79 (4)*	294 ± 32 (4)*	805 ± 132 (4)*	3218 ± 400 (4)

The cells were incubated with [^{14}C]-histamine (100 ng/ml) for 60 min, washed three times and extracted.
Number of experiments in parentheses. Values are means with s.e.
* Statistically significant difference from controls ($P < 0.01$).

Discussion

The present experiments have shown that in the guinea-pig atrium all the antihistamines studied inhibit the accumulation of total radioactivity and the methylation of histamine, without affecting the uptake of the unchanged amine; in mouse mast cells, they simply block the uptake of histamine.

These effects are unrelated to the actions shown by the same drugs in shifting the cumulative dose-response to noradrenaline. Burimamide, metiamide and triprolidine do not potentiate noradrenaline, although they significantly inhibit the accumulation of labelling and

the formation of methyl-histamine. Moreover, cocaine itself, at concentrations capable of increasing the positive chronotropic effect of exogenous noradrenaline failed to modify the uptake and metabolism of histamine, both in the atrium and in the mast cells.

It is possible that in the guinea-pig atrium the decreased formation of methyl-histamine produced by the antihistamines would lead to an equilibrium between the intraceullular and extracellular concentrations of histamine, thus blocking a further accumulation of label in the cells.

In mast cells, which are incapable of metabolizing histamine (Moroni *et al.*, 1972) it is possible that a competition for histamine binding sites by antihistamines would lead to a steady state between intracellular and extracellular histamine, thus blocking the uptake of histamine into the cells.

In our experiments, the inhibition of histamine methylation is shared by H_1- and H_2-receptor antagonists, although they differ in relative potency (burimamide > chlorpheniramine > triprolidine > metiamide). Classical antihistamines have been shown to inhibit histamine methyltransferase from different sources *in vitro* (Netter & Bodenschanz, 1967; Taylor & Snyder, 1972; Barth, Niemeyer & Lorenz, 1973a); the same holds true for H_2-antagonists (Schayer & Reilly, 1973; Barth, Niemeyer & Lorenz, 1973b).

However, no correlation exists between the ability of antihistamines to inhibit histamine methyltransferase activity and their antihistaminic potency, since the concentrations needed to inhibit histamine methylation differ from those required to block cardiac H_1- and H_2-receptors (Table 3).

In conclusion it is evident that some antihistamines are capable of blocking the uptake and metabolism of histamine. The reported antihistaminic potency of these compounds does not correlate with their ability to block histamine uptake and metabolism as determined in these experiments. These effects therefore do not appear to be related to the antihistaminic property *per se* but rather to other properties, which may be tentatively identified as an inhibition of histamine methylating enzymes, or competition at the binding sites for histamine uptake.

Table 3 Comparison of the relative potencies of the antihistamines with their effectiveness in blocking histamine-methylation

	K_D (μM)*	IC_{50} (μM)°
Triprolidine	–	14
Chlorpheniramine	–	5
Burimamide	7.8	0.47
Metiamide	0.92	30

* Receptor drug dissociation constants; data from Black & Spencer (1973). Positive chronotropic action of histamine on guinea-pig atria.

° IC_{50} Concentration of drug which caused 50% inhibition of histamine methylation in the guinea-pig atria. It was evaluated by plotting on probability log-paper the mean percentage inhibition against the molar concentration of the drug.

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