Short communications

The inhibition of histamine uptake and metabolism by burimamide in the guinea-pig atrium and in mouse neoplastic mast cells, *in vitro*.

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Burimamide, an H_2-receptor blocking agent, has been shown capable of blocking the uptake and metabolism of [^{14}C]-histamine, both in the guinea-pig isolated atrium and in murine neoplastic mast cells. This occurs even at concentrations far below the dose range capable of blocking the positive chronotropic effect of histamine in the isolated atrium. In the atrium, very low concentrations of burimamide were found capable of blocking histamine metabolism.

In the guinea-pig isolated atrium histamine can be accumulated by a process requiring both metabolic energy and the integrity of the mast cell population. The bulk of histamine taken up is mainly catabolized into 1-4-methyl-histamine and other metabolites (Moroni, Buiatti & Mannaioni, 1971; Mannaioni, 1972; Moroni & Mannaioni, 1972).

Unlike the atrium, a clone of murine neoplastic mast cells is incapable of metabolizing histamine (Moroni, Buiatti & Mannaioni, 1972), the histamine turnover *in vitro* representing uptake, binding and release. In both preparations, when sympathomimetic amines are present in the perfusion fluid, they compete for histamine uptake (Day & Stockbridge, 1964; Green, 1966; Mannaioni, Fischer & Giarman, 1968; Giotti & Mannaioni, 1968; Moroni *et al.* 1971; Mannaioni, 1972).

At this point it seems to be important to obtain information about the effect of other drugs on histamine uptake and metabolism. Thus burimamide, *N*-methyl-*N'*-(4-4 (5)-imidazolyl) butyl-thiourea, seems to be a drug of choice as it is capable of blocking the positive chronotropic action of histamine in the guinea-pig isolated atrium, whose receptors have been recently classified as histamine H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972).

This paper describes the effect of this drug on the uptake of histamine in preparations in which the accumulation of histamine is mainly due to uptake (mast cells) or to uptake and metabolism (isolated atrium).

Methods.—Preparations used were the isolated, electrically driven (0·5 ms duration; twice threshold voltage; 120 beats/min) guinea-pig left atria incubated in oxygenated Tyrode at 30° C; pH 7·4 (Cervoni, Kirpekar & Schwab, 1966). In each experiment the atria were perfused either with histamine alone or with a medium containing histamine plus various concentrations of burimamide. The muscles were then washed for 10 min to remove extracellular histamine.

Mast cells of the HC line (Furth Mastocytoma) were grown in the ascitic form in LAF_1 mice (Jackson Memorial Laboratory). Cells were removed from the mice at an appropriate phase of the tumour growth by aspiration of the peritoneal fluid. They were then collected by centrifugation, and resuspended in a medium containing histamine, and histamine plus different concentrations of burimamide. After 1 hour's incubation (gas phase: air; pH, 7.45) a cell count was taken; the cells were harvested, washed three times and extracted.

Cardiac tissues and mast cells were homogenized in 0·4 N perchloric acid and suitable aliquots were taken for the assay of total radioactivity, histamine and methyl-histamine. Total radioactivity was measured directly in the perchloric acid extracts, with a liquid scintillation counter (Packard, TriCarb, 3314); histamine was determined by the isotopic dilution (BSH) method (Schayer, 1968), and methylhistamine was assayed according to Snyder, Axelrod & Bauer (1964). In mast cells, assay by the BSH method showed that [^{14}C]-histamine could account for all the radioactivity, so that in further experiments it was no longer necessary to use the isotope dilution procedure, and the amount of radioactive material found in perchloric extracts was directly referred to [^{14}C]-histamine.

Histamine 2-zing[^{14}C] dihydrochloride, specific activity 54 mCi/mmol was purchased from the Radiochemical Centre, Amersham, England ; burimamide dihydrochloride was a gift from Dr. J. W. Black, Smith, Kline & French Laboratories Ltd., to whom we are deeply grateful.

Results.—In the highest concentration used (3×10^{-4}M) burimamide did not affect the histamine level either in the isolated atrium or in the mouse mast cells.

Burimamide can inhibit the accumulation of histamine in both preparations simply by competing for the uptake sites on the cell membrane or by preventing the binding of histamine to intracellular ligands (storage and metabolic sites). If burimamide competes for the uptake sites on the cell membrane, the inhibitory action of the drug on both atria and mast cells should be evident. However this is not the case, as in the atria burimamide inhibits histamine methylation, but does not affect the accumulation of [^{14}C]-histamine. This may indicate that burimamide acts at intracellular levels on both storage and metabolic sites. In the atria, the metabolic sites are prevalent and burimamide inhibits the methylation of histamine ; in the mast cells there are only storage sites and thus burimamide inhibits the accumulation of [^{14}C]-histamine.

TABLE 1. *Inhibition of* [^{14}C]*-Histamine uptake and metabolism by burimamide in the guinea-pig isolated atrium* and murine neoplastic mast cells*** in vitro

Concentration of burimamide (M)	Guinea-Pig left atrium			Mouse mast cells
	Total radioactivity	Me Hist. (d/min × 10^{-3})/g	Hist.	Hist. (d/min)10^{-6} cells
0 (controls)	168·3±10	54·2±2·4	11·7±1·7 (30)	5308±460 (4)
3×10^{-7}	123·6±7	24 ±3·1	9·1±1·6 (4)	—
3×10^{-6}	60 ±8	8·8±0·8	9 ±1·3 (4)	2047±70 (4)
1×10^{-5}	36·3±5	5 ±0·5	8·3±1·5 (4)	1066±63 (4)
3×10^{-5}	27 ±3	7·5±1·3	8·2±1·4 (6)	494±63 (4)
1×10^{-4}	25·6±3·5	<5	9·6±3·5 (4)	215±14 (4)
3×10^{-4}	14·4±3	<5	8 ±1·5 (4)	166±9 (4)

*100 ng/ml; 30 min incubation; 10 min washing. **100 ng/ml; 60 min incubation; 3 washings. No. of experiments in parentheses. Hist=[^{14}C]-histamine; Me hist, [^{14}C]-methyl-histamine.

In the isolated atrium, concentrations of burimamide, ranging from 3×10^{-7} to 3×10^{-4}M, prevented the accumulation of total radioactivity, as well as the formation of methyl-histamine, in a dose-dependent fashion. In the same concentrations, burimamide did not significantly block the uptake of the unchanged amine (Table 1).

In mouse mast cells, burimamide inhibited the accumulation of histamine.

Discussion.—In the guinea-pig heart, histamine is taken up and promptly metabolized, the accumulation of total radioactivity mainly reflecting methylated and other metabolites. On the other hand in the HC line, histamine is taken up actively (Moroni *et al.*, 1972), the differences between guinea-pig atrium and mouse mast cells lying in the inability of mast cells to metabolize the taken-up amine. It is therefore of interest to compare drug effects on histamine uptake in both preparations as, in the atrium, the metabolism of the taken-up amine could be a rate limiting step of the further access of histamine to the intracellular pools. Such is not the case in mast cells, where the rate limiting step would be either the binding to intracellular storage sites or the release.

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