Prostaglandin H Synthetase Activates Xenobiotics into Free Radicals: Histamine Release and Biochemical Characteristics

E.	Emanuela	Masini
E.	Eliana	Giannella
B.	Beatrice	Palmerani
A.	Alessandra	Pistelli
F.	Francesco	Gambassi
P.F.	Pier Francesco	Mannaioni

Department of Preclinical and Clinical Pharmacology, University of Florence, Italy

Abstract

In isolated rat serosal mast cells, studies with the fluorescent Ca^{2+} indicator, quin 2, and with [^3H]-myo-inositol to label endogenous polyphosphoinositides have established that an increase in cytosol Ca^{2+} levels was obligatory for histamine release by free radicals. No substantial breakdown of phosphatidylinositol and related polyphosphoinositides was associated with generation of the Ca^{2+} signal and histamine release, suggesting that the release of histamine by free radicals entails different pathways than the calcium-mobilizing receptors linked to polyphosphoinositides as second messengers.

Correspondence to: Prof. P.F. Mannaioni, Department of Preclinical and Clinical Pharmacology, Viale G.B. Morgagni, 65, I-50134 Firenze (Italy)

Introduction

A variety of widely used xenobiotics such as paracetamol, cocaine and mitomycin C, all known to be activated into free radical species in vivo and in vitro [1], produce the release of histamine from rat serosal mast cells only in the presence of oxidizing systems such as rat liver microsomes or prostaglandin H synthetase (PHS) [2, 3]. The releasing process is mostly exocytotic in nature, and is blocked by anti-free radical interventions such as α -tocopherol, reduced glutathione and d-mannitol, suggesting a free-radical-driven histamine release. The same results were obtained with prostanoid-generating systems [4].

The secretory nature of the releasing process prompted us to study the signal transduction systems in the release of histamine by free radicals, by measuring its relationship with the cytosolic calcium concentration and the hydrolysis of inositol phospholipids.

Materials and Methods

Mast cells were collected by washing the abdominal and thoracic cavities of male albino Wistar rats (200–250 g) and purified through gradient centrifugation by means of a Beckman elutriator. Aliquots of cells ($4\text{--}5 \times 10^6$ /sample) were suspended in a buffered salt solution of the following composition: 145 mM NaCl, 0.9 mM CaCl_2 , 2.4 mM KCl, 0.45 mM MgCl_2 , 0.1% glucose, 0.1% human

serum albumin (Behringwerke, Marburg/Lahn, FRG), adjusted to pH 7.4 with 10% Sørensen phosphate buffer. The cells were incubated for 30 min at 37 °C in the solution described above with various free-radical-generating systems such as xanthine (0.5 mM)/ xanthine oxidase (20 mU/ml), FeCl_3/ADP (10 μM /100 μM) and the combination of paracetamol (10 $^{-5}$ M) and PHS

(1,000 mU). After incubation the reaction was stopped by chilling the tubes, and the cells were harvested by centrifugation.

Histamine was measured fluorometrically [5] and histamine release (supernatant histamine) was expressed as a percentage of the total present in the cells plus supernatant. Spontaneous histamine release ranged between 1 and 8% and was subtracted from all values.

Mast cells originating from the same aliquot were loaded with 50 μ M quin 2AM, placed in a quartz cuvette at 37 °C under constant stirring, and the fluorescence measured at 492 nm with excitation at 339 nm using a Perkin Elmer spectrofluorometer (model LC85). Fluorescence was continuously recorded, and secretagogues added to the cells without any interruption of the recording. For assays of inositol phosphates, inositol phospholipids were radiolabeled with myo-2-[3H]-inositol (4 μ Ci/ml). The cells were disrupted by sonication, centrifuged and the [3H]-inositol phosphates were assayed by means of a Packard scintillation counter, after separation on Dowex-c column chromatography [6].

Results

The data shown in table 1 indicate that the relationship between $[Ca^{2+}]_i$ and histamine release for A23187 or the free-radical-generating systems differs widely.

In fact, no significant histamine release was ob-

Free-Radical-Induced Histamine Release

135

Table 1. Calcium and histamine secretion of rat serosal mast cells induced by different stimuli

Stimulus	Maximal increase $[Ca^{2+}]_i$, nM	Histamine release, %
A23187		
0.1 μ M	182 $\pm$ 31	5.6 $\pm$ 1.3 (2)
1 μ M	678 $\pm$ 149	32.3 $\pm$ 2.6 (3)
Xanthine/xanthine oxidase		
0.5mM/20mU/ml	221 $\pm$ 16	28.5 $\pm$ 3.4 (2)
FeCl ₃ /ADP		
10 μ M/100 μ M	530 $\pm$ 82	74.3 $\pm$ 5.9 (4)

diylinositol was associated with the generation of the calcium signal.

The uncoupling of the calcium signal from phosphatidylinositol breakdown suggests that the secretion of mast cell histamine is not driven by free radicals acting upon calcium-mobilizing receptors using diacylglycerol and/or inositol-1,4,5-triphosphate as putative Ca^{2+} mobilizers. The coupling of the secretion of histamine with the increase in cytosol Ca^{2+} may be tentatively explained either through the free-radical-operated activation of the cAMP pathway, or by a regulation of a Ca^{2+} gate at the plasma membrane.

Itained in response to concentrations of A23187 (0.1 μ M) which gave $[Ca^{2+}]_i$ increases (182 nM) similar to those induced by xanthine/xanthine oxidase (221 nM), causing an appreciable amount of histamine secretion. On the other hand, a close relationship was present between $[Ca^{2+}]_i$ and the release of histamine within the two different free-radical-generating systems. Interestingly, the hydrolysis of inositol phospholipid was clearly stimulated by compound 48/80 in close relationship with the release of histamine, while a free-radical-generating system (paracetamol plus PHS) failed to stimulate the phosphatidylinositides breakdown, while activating histamine secretion (data not shown).

Discussion

Studies with the fluorescent Ca^{2+} indicator, quin 2, have established that the secretion of mast cell histamine driven by free-radical-generating systems is coupled with an increase in cytosolic calcium concentration. The increase in cytosolic Ca^{2+} levels (from 221 to 530 nM when cells were maximally stimulated) was a dynamic response, since in all experiments histamine release was correlated with the magnitude of the calcium signal. However, no breakdown of phosphatidylcholine was observed.

Acknowledgement
These investigations were supported by a grant from MPI (60%), University of Florence.

References

Mannaioni PF, Giannella E, Palmerani B, et al: Free radicals as endogenous histamine releasers. *Agents Actions* 1988;23:129–142.

Masini E, Lodovici M, Fantozzi R, et al: Histamine release by free radicals: Paracetamol-induced histamine release from rat peritoneal mast cells after in vitro activation by monooxygenase. *Agents Actions* 1986;18:85–88.

Masini E, Giannella E, Bani Sacchi T, et al: Histamine release from serosal mast cells by intermediate products of arachidonic acid metabolism. *Agents Actions* 1987;20:202–205.

Masini E, Palmerani B, Bani Sacchi T, et al: Mast cell histamine release induced by intermediate products of arachidonic acid metabolism. *Int Arch Allergy Appl Immunol* 1987;82:279–282.

Blandina P, Fantozzi R, Mannaioni PF, et al: Characteristics of histamine release evoked by acetylcholine in isolated rat mast cells. *J Physiol* 1980;301:281–293.