Role of Serine Esterases in the Activation of Rat Peritoneal Mast Cells

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Key Words

Serine esterases

Mast cells

Mediator release

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It has been suggested that activation of a chymotryptic protease may be involved in stimulus-secretion coupling in the mast cell [1]. Accordingly, a number of inhibitors of, or substrates for, this enzyme can prevent mediator release from different mast cell phenotypes and basophil leucocytes [2, 3]. These inhibitors are only effective if present at the time of cell stimulation, suggesting that the enzyme is normally found in the form of a zymogen which is converted to the active entity following IgE receptor cross-linking. We have now investigated this hypothesis further and have examined the effect of chymotryptic inhibitors and substrates in normal and permeabilized rat mast cells. In addition, we have monitored the hydrolysis of an entrapped fluorescent substrate following cell activation.

Rat peritoneal mast cells were recovered by direct lavage and purified by density gradient centrifugation over Percoll [4]. Where appropriate, agents were introduced into the cell cytosol after reversible permeabilization with ATP [5]. His-tamine release was measured as before [4]. In fluorescence experiments, exocytosis was prevented by incubation of the cells with antimycin A (20 min, 1 μ M). Cells (2×10^5 /ml) were permeabilized, preloaded with the substrate N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methyl-coumarin (S-Pep-MCA, Sigma) and incubated in a thermo-stated (37°C) quartz cuvette (5 mm path length) of a Perkin-Elmer luminescence spectrometer fitted with an electronic stirrer. The sample was illuminated at an excitation wavelength of 380 nm, and emitted light was recorded at 460 nm. Secretagogues were added in a minimum volume as indicated.

Anti-rat IgE (ICN Immunologicals) and substance P (Neosystem) were used as secretory stimuli. The general serine enzyme inhibitor phenylmethylsulfonyl fluoride (PMSF), the suicide inactivator isatoic anhydride, the microbial product chymostatin, and the chymotryptic substrates N-acetyl-L-tyrosine ethyl ester (ATEE) and 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC) were obtained from Sigma. All values are given as means $\pm$ SEM for the number of experiments noted.

Immunologic histamine release from rat mast cells was modestly inhibited by all of the test compounds. However, the activity of these agents was markedly increased when they were directly introduced into the cells by permeabilization with ATP (table 1). In contrast, the

compounds had no effect against secretion induced by substance P (table 1) and were also inactive against mastoparan and compound 48/80 (data not shown). Immunologic stimulation of mast cells loaded with S-Pep-MCA led to a pronounced and rapid increase in the fluorescence, indicating activation of a pro-enzyme and hydrolysis of the substrate (fig. 1a). However, compound 48/80 produced no change in fluorescence (fig. 1b). The current study then provides strong evidence for the involvement of a chymotryptic protease in IgE-mediated activation of rat mast cells. This process appears to be specific for immunologic stimulation and does not seem to be involved in secretion induced by polyamines such as compound 48/80 and substance P. Identification of the enzyme may then provide a new therapeutic target for the modulation of mast cell function.

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Table 1. Inhibition of histamine release from normal and permeabilized rat peritoneal mast cells

Cells were preincubated (10 min) with the test agents (100 μ M, except PSMF 1 mM) or the latter were introduced directly into the cytosol by permeabilization with ATP [5]. Cells were stimulated with anti-rat IgE (200-fold dilution, control release $33.5 \pm 3.0\%$) or substance P (50 μ M, control release $46.0 \pm 3.0\%$). For abbreviations, see text. Values are means $\pm$ SEM for four experiments.

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60

40-

20

S o

‡ Anti-IgE

0.0

0.5

¢ 40[]

20-

1.0 1.5 2.0 2.5

‡ Compound 48/80

0.0

0.5

2.0

2.5

1.0 1.5

Time (min)

Fig. 1. Fluorescence changes in permeabilized rat peritoneal mast cells containing the chymotryptic substrate S-Pep-MCA. Anti-IgE (250-fold dilution) (a) or compound 48/80 (b) (1

µg/ml) were added at the points indicated. Traces are representative of a least 4 similar recordings in each case.

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