Definition of Platelet-Derived Histamine-Releasing Factor and Histaminergic Receptors Modulating Platelet Aggregation

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Abstract

Endogenous and exogenous free radical scavengers significantly decrease mast cell histamine release induced by coincubation with resting, activated platelets or with platelet-derived supernatant. Histamine and pyridylethylamine dose-dependently enhance platelet aggregation; the effect is potentiated by ranitidine and blocked by mepyramine. Histamine increases also cytosolic calcium concentration in platelets stimulated with thrombin, and binding sites for [³H]-mepyramine are present on platelet membranes.

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Platelets may behave as inflammatory cells. In fact, they generate superoxide anions and carry a membrane-bound hydrogen peroxide-generating system which is activated by platelet agonists and immunological stimuli. Moreover, human basophil histamine release is induced by substances released from stimulated human platelets [1], and coincubation of rat mast cells with human platelets leads to a significant release of histamine [2]. In turn, histamine enhances platelet aggregation induced by different agonists [2].

In the present experiments we attempted to ascertain a free radical mechanism in the release of mast cell histamine induced by platelet-derived histamine-releasing factors and to identify histaminergic receptors on platelet membranes.

The release of histamine from purified rat serosal mast cells was measured fluorimetrically after: (1) coincubation with resting human platelets; (2) coincubation with platelets aggregated with arachidonic acid (1.43×10^{-5} M) or thrombin (40 mU/ml), and (3) incubation with supernatant derived from arachidonic acid-aggregated platelets. Binding studies with [³H]-mepyramine were performed to identify the presence of histaminergic receptors on platelet membranes.

Free radical scavengers or pharmacological interventions meant to neutralize free radicals significantly diminished the release of histamine from mast cells induced by coincubation with resting and activated platelets aggregated by a variety of agents.

In the same experimental setting, lipoperoxidation of mast cell membranes was demonstrated by the generation of malonyldialdehyde and conjugated dienes. Histamine dose-dependently enhanced in vitro platelet aggregation induced by a variety of platelet agonists; this effect was mimicked by pyridylethylamine and reduced by mepyramine, while compounds acting at H₂ receptors were inactive. Moreover, an increase of intracellular calcium induced by histamine

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during platelet aggregation was demonstrated using fura-2AM as fluorescent probe. Specific binding sites for [3H]-mepyramine were present in membrane preparations of human platelets. Scatchard plot analysis revealed a homogeneous population of specific binding sites with a Bmax of 701 fmol/10⁷ cells.

The present results suggest that resting and activated platelets induce the release of histamine from mast cell by generating free radical species. Histamine in turn potentiates platelet aggregation by increasing cytosolic free calcium in a way which can be modulated by the activation of $\frac{3}{8}$ receptors present on platelet membranes.

References

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