The effects of G-protein activators, mastoparan and compound 48/80, on serotonin secretion and signaling pathway in human platelets

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Abstract

Mastoparan and compound 48/80 have been found to accelerate guanine nucleotide exchange and GTPase activity of purified GTP-binding protein. These compounds can directly activate secretory processes of mast cells, pancreatic islets and adrenal chromaffin cells by penetrating through plasma membranes and directly stimulate membrane GTPase activity and stimulates PLC-mediated events without mediating via receptor binding. This study aims to examine whether these compounds affect secretion of both intact and permeabilized human platelets, and examine the subtype of G-protein signaling. [^{3}H]-serotonin labeled platelets were pre-incubated for 5 min and were activated with various concentrations of mastoparan and compound 48/80 for 3 min at room temperature or preincubated with streptolysin O (SLO) for 2 min before activation. The amounts of [^{3}H]5-HT release were determined by liquid scintillation counting. Mastoparan was found to produce a concentration-dependent increase in 5-HT release from intact platelets with an EC_{50} of 20 μ M.. The maximal secretion was obtained at the concentration of 60 μM.. Similarly, compound 48/80 caused a concentration-dependent increase in 5-HT release with maximal secretion obtained at the concentration of 400 μg/ml.. Permeabilized platelets with streptolysin O significantly increase serotonin secretion. To investigate whether the observed stimulation of serotonin secretion is mediated through the G_i subunit of G-protein, the G-protein blocking agents (e.g. G_i-sensitive pertussis toxin, benzalkonium chloride, a selective G_i inhibitor, and daunomycin, a lipid bilayer stabilizer) were used. Mastoparan- and compound 48/80-induced secretion was inhibited by preincubation with pertussis toxin only in SLO-permeabilized platelets whereas benzalkonium chloride and daunomycin did not affect mastoparan- and compound 48/80-induced secretion in both intact and SLO-permeabilized platelets. The results from this study suggested that mastoparan- and compound 48/80 promoted secretion by mechanisms involved neither the stimulation of G_i-subtype of G-protein nor interfering with lipid bilayer of the membranes. The secretory event may result either from a direct fusogenic action and/or from the stimulation of putative exocytosis-linked G-protein, G_e.. Their mechanisms on small GTPase proteins, on G_e and on membrane perturbation in human platelets remain to be elucidated.

Keywords : G-protein activators, serotonin secretion, signaling pathway, human platelets