A GTP Analogue Induces Calcium Release but Not Secretion in Rat Mast Cells

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Abstract

Two G protein-mediated events, calcium release and secretion, were measured in single rat peritoneal mast cells using the patch-clamp technique. Various phosphorothioate analogues of GTP were introduced into the cells. While GTP γ S and Rp-GTP α S activated both processes, Rp-GTP β S was found to induce repetitive calcium release in the absence of exocytosis. This response was modulated by IP $_3$, heparin, compound 48/80, and ATP. Our results suggest that Rp-GTP β S simulates G $_p$, which links receptors to calcium release.

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Rat peritoneal mast cells were obtained by peritoneal lavage and Percoll gradient centrifugation [1]. They were cocultured with Swiss 3T3 fibroblasts [2] and used within 5 days. For experiments, the cells were bathed in magnesium-rich Ringer (in mM: NaCl, 140; KCl, 2.5; MgCl $_2$, 5; CaCl $_2$, 2; NaOH-Hepes, 10; pH 7.2). The whole-cell configuration of the patch-clamp technique was used to load the cells with internal solution (in mM: K-glutamate, 145; NaCl, 8; MgCl $_2$, 1; KOH-Hepes, 10; Mg-ATP, 0.5), supplemented with the calcium indicator Fura-2 (100 μ M), GTP analogues (200 to 500 μ M) (kindly provided by F. Eckstein), IP $_3$, and ATP as indicated in the text. Fura-2 fluorescence [3] was used to determine the intracellular free calcium. The cell membrane capacitance was measured as an indicator of exocytosis [4].

GTP γ S irreversibly activates cellular G proteins. When this analogue is added to the pipette solution, the intracellular calcium rises transiently some 30 s after breakin, and the capacitance increases, indicating degranulation. This has been shown to be due to at least two G proteins, G $_p$ and G $_q$; G $_p$ liberates calcium via the phosphoinositide (PI) pathway while G $_q$ is thought to stimulate exocytosis [5, 6].

The analogue Rp-GTP α S induces a similar, though less pronounced, response to GTP γ S. The ana-

logue Rp-GTP β S, on the other hand, produces oscillatory calcium release (fig. 1) but no significant capacitance increase. The Sp isomers were much weaker in their effects.

The Rp-GTP β S-induced calcium spikes were studied more closely. The involvement of the PI pathway in the response was examined by coapplying a high dose of IP $_3$ (10 μ M) which presumably empties most of the IP $_3$ -sensitive stores. This prevented the calcium transients. Heparin (250 μ g/ml), which blocks IP $_3$ receptors [7], also inhibited the oscillations. Compound 48/80, which mobilizes calcium by way of the PI pathway, was found to accelerate Rp-GTP β S-induced calcium oscillations. Thus the PI pathway is involved in the Rp-GTP β S response. The oscillations were modulated by the intracellular ATP concentration. This could be shown by varying the ATP concentration in the pipette solution. Increasing the cellular ATP from 0.5

mM to 3.8 mM decreased the latency to the first transient, and increased the number of transients and their frequency.

In summary, the GTP analogue Rp-GTP β S selectively stimulated one of two examined G protein-mediated mast cell events. The differential activation of calcium release suggests a preferential interaction of Rp-GTP β S with G α . Rp-GTP β S might be a useful probe in the study of this G protein in mast cells.

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Fig. 1. Effects of Rp-GTP β S on free intracellular calcium. The pipette solution contained 0.5 mM ATP and 200 μ M Rp-GTP β S.

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