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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 analgoues of GTP were introduced into the cells. While GTPγS and Rp-GTPαS activated both processes, Rp-GTPβS 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-GTPβS simulates G_p, which links receptors to calcium release.

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 m*M*: 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 m*M*: 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].

GTPγS 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_e; G_p liberates calcium via the phosphoinositide (PI) pathway while G_e is thought to stimulate exocytosis [5, 6].

The analogue Rp-GTPαS induces a similar, though less pronounced, response to GTPγS. The analogue Rp-GTPβS, 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-GTPβS-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 μg/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-GTPβS-induced calcium oscillations. Thus the PI pathway is involved in the Rp-GTPβS 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 m*M* to 3.8 m*M* decreased the latency to the first transient, and increased the number of transients and their frequency.

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

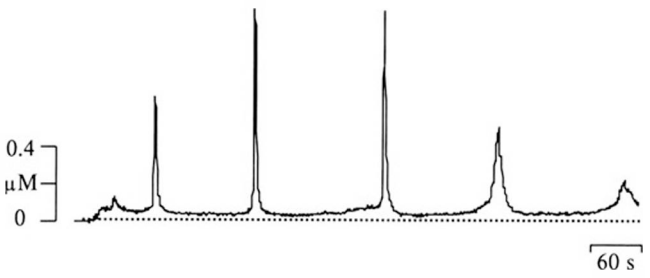

Fig. 1. Effects of Rp-GTPβS on free intracellular calcium. The pipette solution contained 0.5 m*M* ATP and 200 μ*M* Rp-GTPβS.

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