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Modulation of calcium mobilization by guanosine 5′-*O*-(2-thiodiphosphate) in *Xenopus* oocytes

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We investigated the effect of intracellular loading of the non-hydrolyzable guanosine 5′-diphosphate analogue GDPβS on calcium mobilization by IP_3 and on calcium influx in *Xenopus* oocytes. Assayed by two electrode voltage-clamp recording, GDPβS-loaded oocytes demonstrated a marked augmentation of the fast component of the response to IP_3 injection, an attenuation of the slow component and an increase in membrane calcium permeability. These effects on calcium mobilization suggest that GDPβS may facilitate calcium translocation both across the plasma membrane and between different intracellular calcium pools.

Inositol 1,4,5-trisphosphate; Calcium pool; Calcium channel

1. INTRODUCTION

Intracellular calcium oscillations and the magnitude and duration of the cell's response to calcium-mobilizing transmitters may be modulated by a complex orchestration of release mechanisms from the IP_3-sensitive and IP_3-insensitive calcium stores and by calcium translocation between these stores [1]. Recent experiments suggest that the IP_3-sensitive and IP_3-insensitive calcium pools may exist in dynamic equilibrium. The chemicals, GTP [2,3], IP_4 [4,5], and IP_3 [6] have all been implicated in regulating the exchange of calcium between the pools.

Xenopus oocytes allow intracellular application of substances and monitoring of changes in $[Ca^{2+}]_i$ via the calcium-sensitive chloride current [8]. Intracellular IP_3 injection elicits a well-characterized two-component response due to calcium activation of the oocyte's chloride channel [7–9]. During experiments on G-protein function in oocytes, we noticed that this response to IP_3 was dramatically altered by loading the cells with the non-hydrolyzable guanosine 5′-diphosphate analogue GDPβS. Despite the reported lack of effect of this compound in permeabilized cells [10,11], GDPβS appeared to alter the size of the IP_3-sensitive calcium store in intact oocytes. We proceeded, therefore, to study the effect of GDPβS preloading on the fast and slow components of the response to IP_3. In the course of this work we unexpectedly found that GDPβS preloading also leads to an increase in membrane calcium permeability. Thus it appears that GDPβS facilitates calcium translocation between different pools.

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; G-protein, guanyl nucleotide binding protein; IP_4, inositol 1,3,4,5-tetrakisphosphate; GDPβS, guanosine 5′-*O*-(2-thiodiphosphate); ADPβS, adenosine 5′-*O*-(2-thiodiphosphate); EGTA, ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid

2. MATERIALS AND METHODS

GDPβS was obtained from Boehringer-Mannheim (Indianapolis, IN). Collagenase type 1A and other reagents and chemicals were obtained from The Sigma Chemical Co. (St. Louis, MO).

2.1. *Oocyte culture and electrophysiology*

Oocytes were maintained and recorded as previously described [12]. The calcium-free medium for oocyte incubation and perfusion during experiments contained 86 mM NaCl, 2 mM KCl, 15 mM $MgCl_2$, 0.2 mM EGTA and 5 mM Hepes, pH 7.5. For application of calcium while recording, 5 mM $CaCl_2$ was substituted for 5 mM $MgCl_2$ (no EGTA).

2.2. *Intracellular application of substances*

For acute IP_3 injection, a pressure injection system was used. This is subsequently referred to as 'injection'. For this purpose, a cell which was under voltage-clamp had a third micropipette introduced. IP_3 was delivered by metered pulses of compressed nitrogen (Picospritzer II, General Valve Corporation, Fairfield, NJ). Before insertion into and after withdrawal from the cell, the pipette tip was immersed in a drop of mineral oil and the droplet size was measured with an eye piece micrometer. Cells were injected with a volume less than 0.4% of the volume of the oocyte (100–200 μm diameter). For loading the cell with GDPβS a pulled glass capillary tube broken to a 10 μm diameter tip and a microdispenser were employed (Drummond, Broomall, PA). The cells were penetrated at the animal pole and 30–50 nl of solution was introduced. This is referred to as 'preloading'.

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2.3. *Monitoring of intracellular calcium concentration*

Direct measurement of calcium concentration via fluorescent dyes [8,13] or calcium sensitive microelectrodes [14] demonstrates that IP_3 leads to an increase in $[Ca^{2+}]_i$ in oocytes. Increases in $[Ca^{2+}]_i$ activate the oocyte's calcium-sensitive chloride current [15,16] which we therefore used to monitor the changes evoked by IP_3.

3. RESULTS

Oocytes were preloaded with 0.5–2.0 mM of GDPβS (trilithium salt) and incubated for 1–2 h in calcium-free medium. These experiments were done in calcium-free medium because GDPβS preloaded cells deteriorate in calcium-containing medium due to an increase in membrane calcium permeability (vide infra). Preloading oocytes with an equivalent dose of lithium chloride induced no change in their response to IP_3 injection. The typical, well-characterized response to 0.5–1.0 pmol IP_3 injection [7–9] was obtained with a fast-transient component and a prolonged slow component lasting several minutes and usually accompanied by oscillations (Fig. 1A,C). GDPβS preloading led to a marked increase in the amplitude and duration of the fast response to 0.5–1.0 pmol IP_3 injection. The slow component, however, was absent or nearly absent (Fig. 1B,D). In addition, when oscillations existed in response to IP_3 injection, a decrease in the frequency of current fluctuations in the GDPβS preloaded oocytes was noted (Fig. 1D). The effect of ADPβS preloading on the IP_3 response was also examined. ADPβS led to a marked decrease in the size of the IP_3 responses. In many cases the cells deteriorated following IP_3 injection. We suspect that ADPβS interferes with the cell's ability to pump calcium, leading to depletion of calcium stores, and that the cell was unable to resequester released calcium, causing cell deterioration following IP_3 injection.

For statistical analysis, the peak clamp current of the fast component of the response to IP_3 injection was measured from the baseline. The amplitude of the slow component was measured in reference to a baseline determined before and after the appearance of the slow response, as illustrated in Fig. 1. In lithium preloaded cells, the fast phase amplitude was 262 ± 19 nA (n = 38). GDPβS preloading led to an increase in the fast phase to 706 ± 92 (n = 34, $P < 0.001$, Student's t-test). The amplitude of the second component of the response to IP_3-injection following lithium preloading was 57 ± 20 nA (n = 16). In 2 GDPβS preloaded cells the late component amplitude was less than 10 nA and in 12 cells it was absent (Fig. 1).

We noticed that keeping GDPβS preloaded cells in calcium-containing medium for longer than one hour led to marked deterioration in the cell's membrane potential and resistance. However, when incubated in calcium-free solution, the toxicity of GDPβS preloading was attenuated. We suspected that this calcium-mediated toxicity was due to an increase in

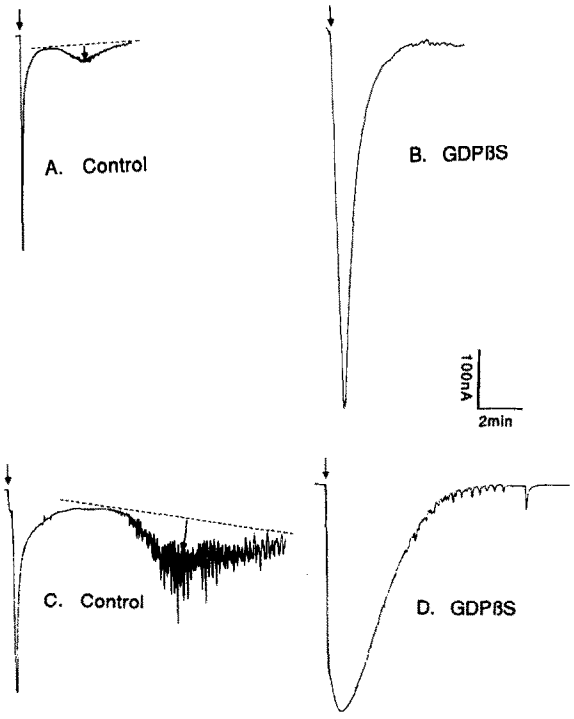

Fig. 1. Effect of GDPβS preloading on response to IP_3 injection. (A, C) Control cells, loaded with 50 nl 1.5 mM lithium chloride injected with 0.5 pmol IP_3. (B, D) Cells loaded with 50 nl 0.5 mM GDPβS injected with 0.5 pmol IP_3.

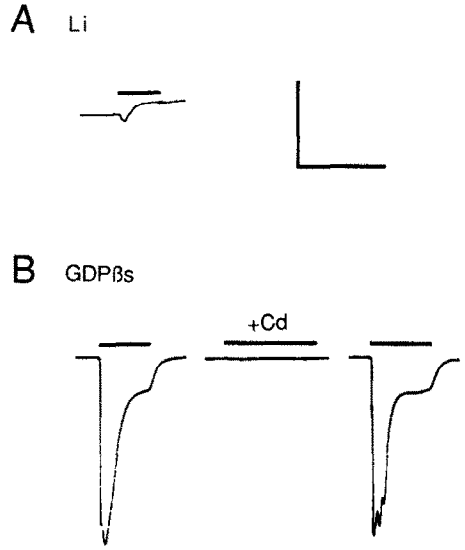

Fig. 2. Extracellular calcium-evoked current in GDPβS preloaded oocytes. (A) Control cell preloaded with 50 nl 60 mM lithium chloride (nominal final concentration 3 mM), incubated for 1.5 h in calcium-free medium, voltage-clamped at −70 mV and exposed to 5 mM calcium-containing perfusion for the time indicated by the horizontal bars. (B) Oocyte was loaded with a nominal concentration 1 mM GDPβS and incubated and exposed to calcium as in the control cell. Antagonism of the response to extracellular calcium by 2 mM cadmium was reversed following washout of the cadmium. Calibration: vert., 100 nA; horiz., 20 s.

membrane calcium permeability induced by GDPβS preloading and therefore examined the effect of acute exposure to calcium-containing medium in GDPβS preloaded cells. Cells were preloaded with lithium chloride or GDPβS as above and maintained for 1–2 h in calcium-free medium. Cells were then voltage-clamped at −70 mV and briefly (5–10 s) exposed to calcium-containing perfusate. In GDPβS preloaded cells a large depolarizing current of 301 ± 16 nA (n = 29) was obtained (Fig. 2). Negligible current was obtained in lithium preloaded control cells (0–20 nA, n = 21). The GDPβS induced calcium permeability was sensitive to inorganic calcium channel blockers, being antagonized by 2 mM cadmium (percent inhibition 87 ± 8%; n = 6, Fig. 2), 2 mM cobalt (81 ± 6%; n = 5) and 2 mM manganese (88%, n = 1). The increase in membrane calcium permeability was not specific to GDPβS preloading and was found after ADPβS preloading as well.

4. DISCUSSION

We find in GDPβS preloaded oocytes that the fast component of the response to IP_3 injection is dramatically augmented and that the slow phase is attenuated. The fast component of the response to IP_3 in the oocyte is generated by calcium release from IP_3-sensitive stores while the slow component is proposed to be due to a calcium-dependent calcium release mechanism [9,17]. As our experiments were performed in calcium-free medium, the contribution of calcium entry to the second phase of the IP_3-evoked response is excluded. One explanation for the effect of GDPβS on calcium mobilization would be that GDPβS causes calcium to move from the IP_3-insensitive to the IP_3-sensitive pool. In this model, illustrated in Fig. 3, the first component of the response to IP_3 represents direct release from the IP_3-sensitive calcium pool and the slow component represents the effect of this calcium in causing calcium release from the IP_3-insensitive pool. Following GDPβS preloading, the pools are fused and IP_3 can empty both pools directly. Thus the first component to IP_3 injection is augmented and the second attenuated.

Some clues to the mechanism of the GDPβS action may be inferred from the studies of calcium translocation in permeabilized cells. In permeabilized cells, hydrolyzable GTP promotes the exchange of calcium between the IP_3-insensitive and the IP_3-sensitive pools [3,11]. Permeabilization with saponin or digitonin allows penetration of IP_3 into the cell. However, as the maximal effect of GTP occurs at concentrations well below the GTP concentration in the intact cell, it seems as if the permeabilization itself serves to unmask the capacity for GTP-inducible calcium translocation, a capacity that was somehow held in check prior to permeabilization [11,18]. Thomas has proposed the existence of a factor which inhibits calcium translocation which is inactivated by permeabilization [11]. His data suggest that, following permeabilization, the cells' own GTP, before diffusing away, can contribute to calcium movement between pools. The long preincubation required for a GDPβS effect in oocytes and the lack of any reported effect of GDPβS on the IP_3-releasable calcium pool in permeabilized mammalian cells [10,11] suggest that GDPβS could be acting indirectly in the oocyte. The effect of GDPβS preloading in the oocyte may be analogous to that of permeabilization in mam-

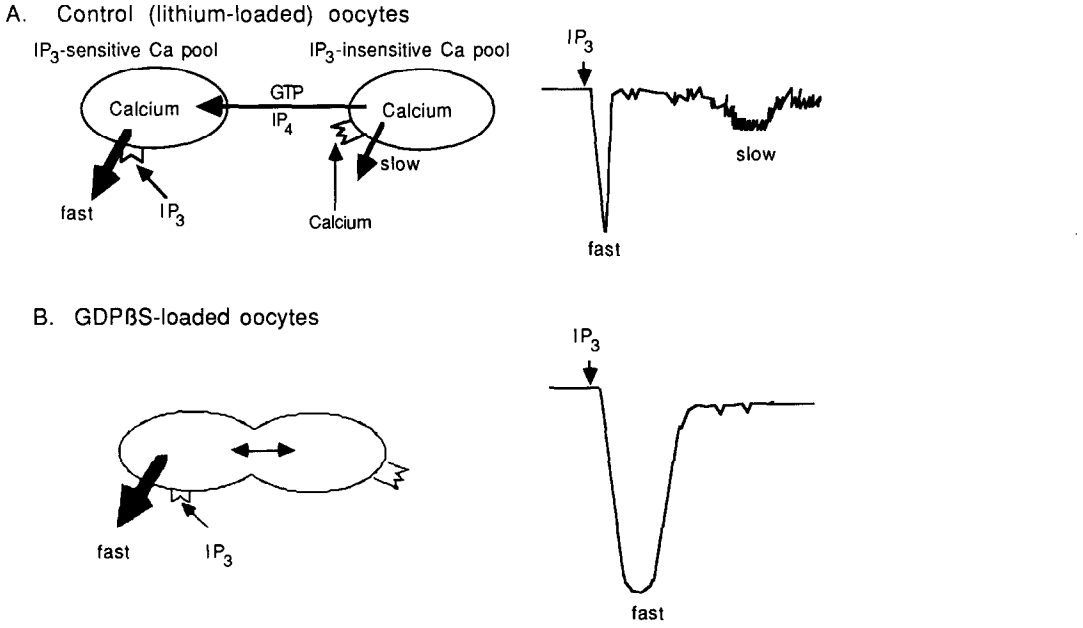

Fig. 3. Proposed mechanism of GDPβS effect on the two-component response to IP_3. The tracings are drawn by hand to illustrate the hypothesis.

malian cells. Perhaps GDPβS disinhibits GTP-mediated calcium movement, thus allowing it to proceed, fueled with the cell's own GTP.

GDPβS also led to an increase in plasma membrane calcium permeability which was sensitive to inorganic antagonists. The mechanism by which the membrane calcium flux is increased by thiophosphate nucleotide preloading is unknown. In both the phenomena we describe, however, GDPβS appears to cause the opening of calcium gates, allowing the transfer of calcium across membranes.

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