Effect of Inositol 1,3,4,5-Tetakisphosphate on Inositol Trisphosphate-activated Ca\(^{2+}\) Signaling in Mouse Lacrimal Acinar Cells*

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In mouse lacrimal acinar cells, microinjection of the metabolically stable analog of inositol 1,4,5-trisphosphate, inositol 2,4,5-trisphosphate ((2,4,5)IP\(_3\)), stimulated both intracellular Ca\(^{2+}\) mobilization and Ca\(^{2+}\) entry. Microinjection of inositol 1,3,4,5-tetakisphosphate ((1,3,4,5)IP\(_4\)), the inositol 1,4,5-trisphosphate-3-kinase product, was ineffective at mobilizing intracellular Ca\(^{2+}\) or activating Ca\(^{2+}\) entry. In lacrimal cells previously microinjected with submaximal levels of (2,4,5)IP\(_3\), the subsequent microinjection of low to moderate concentrations of (1,3,4,5)IP\(_4\), did not result in additional release of intracellular Ca\(^{2+}\), nor did it potentiate the Ca\(^{2+}\) entry phase attributable to (2,4,5)IP\(_3\). However, as previously demonstrated (Bird, G. S. J., Rossier, M. F., Hughes, A. R., Shears, S. B., Armstrong, D. L., and Putney, J. W., J. r. (1991) Nature 352, 162-165), additional injections of (2,4,5)IP\(_3\) induced further mobilization of intracellular Ca\(^{2+}\) and increased the elevated and sustained Ca\(^{2+}\) entry phase. Introduction of high concentrations of (1,3,4,5)IP\(_4\) appeared to inhibit or block the (2,4,5)IP\(_3\)-induced Ca\(^{2+}\) entry phase. These results were consistent with the observed effect of (1,3,4,5)IP\(_4\) in permeabilized lacrimal cells, where (1,3,4,5)IP\(_4\) did not release cellular Ca\(^{2+}\) but at high concentrations inhibited the ability of submaximal concentrations of (2,4,5)IP\(_3\) to release Ca\(^{2+}\). Likewise, injection of a high concentration of (1,3,4,5)IP\(_4\) prior to injection of (2,4,5)IP\(_3\) blocked both release and influx of Ca\(^{2+}\). The inhibitory action of (1,3,4,5)IP\(_4\) on Ca\(^{2+}\) signaling observed in intact cells occurred at concentrations that might be obtained in agonist-stimulated cells. However, in permeabilized cells, (1,3,4,5)IP\(_4\) inhibited Ca\(^{2+}\) mobilization at concentrations exceeding those likely to occur in agonist-stimulated cells. These results suggest that physiologically relevant levels of (1,3,4,5)IP\(_4\) in the cell cytoplasm do not release Ca\(^{2+}\), nor do they potentiate inositol trisphosphate-induced Ca\(^{2+}\) entry across the plasma membrane. Rather, the possibility is raised that (1,3,4,5)IP\(_4\) or one of its metabolites could function as a negative feedback on Ca\(^{2+}\) mobilization by inhibiting inositol 1,4,5-trisphosphate-induced Ca\(^{2+}\) release.

In many cell types, surface receptor activation results in a complex, biphasic Ca\(^{2+}\) response composed of an initial mobilization of internally stored Ca\(^{2+}\), followed by entry of extra-

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1 The inositol phosphates are abbreviated according to the "Chilton Convention" (24), as, for example, (1,4,5)P\(_3\) for di-myoinositol 1,4,5-trisphosphate.
5 mM glutamine, 50 units/ml penicillin, and 50 units/ml streptomycin. The cells were then allowed to attach to glass coverslips coated with Matrigel (Collaborative Biomedical Products, Bedford, MA). Acinar cells were incubated on the glass coverslips for at least 30 min before Ca\textsuperscript{2+} mobilization was initiated by the addition of 3 mM-MgATP. Cell content of \[\text{Ca}^{2+}\] (10 mM pipette concentration, final cellular concentration, 100–200 μM (11)), did not mobilize intracellular Ca\textsuperscript{2+} (\[Ca^{2+}_{\text{i}}\]) nor did it promote Ca\textsuperscript{2+} entry into the cell on restoring the extracellular Ca\textsuperscript{2+} (Fig. 1). Further, the injection of (1,3,4,5)IP\textsubscript{4} did not prevent the ability of thapsigargin to activate \[Ca^{2+}_{\text{i}}\] entry in these cells (Fig. 1). As shown previously in lacrimal cells (11), microinjection of submaximal concentrations of the metabolically stable (1,4,5)IP\textsubscript{3} analog, (2,4,5)IP\textsubscript{3} (1 mM pipette concentration, final cellular concentration 10–20 μM), resulted in a submaximal Ca\textsuperscript{2+} release and a submaximal level of Ca\textsuperscript{2+} entry (Fig. 2a). Subsequent microinjection of additional (2,4,5)IP\textsubscript{3} released additional Ca\textsuperscript{2+} and further increased the level of Ca\textsuperscript{2+} entry (Fig. 2a). Control injections not containing an inositol phosphate did not modify the second Ca\textsuperscript{2+} entry phase as compared with the first (Fig. 2b).

Although it is apparent from these results and our earlier report (11) that (1,4,5)IP\textsubscript{3} provides both a necessary and sufficient signal for both intracellular Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} entry, our previous study did not directly address the possibility that (1,3,4,5)IP\textsubscript{4} might modulate or augment IP\textsubscript{3}-induced Ca\textsuperscript{2+} signaling in intact lacrimal cells. In Fig. 1, c and d, the effect of (1,3,4,5)IP\textsubscript{4} on cells previously microinjected with submaximal concentrations of (2,4,5)IP\textsubscript{3} was examined to determine if (1,3,4,5)IP\textsubscript{4} could either mobilize additional intracellular Ca\textsuperscript{2+} or potentiate the Ca\textsuperscript{2+} entry phase induced by (2,4,5)IP\textsubscript{3} alone.

![Fig. 1. The effect of microinjected (1,3,4,5)IP\textsubscript{4} on Ca\textsuperscript{2+} signaling in a single mouse lacrimal acinar cell. Lacrimal cells were incubated in a nominally Ca\textsuperscript{2+}-free medium, microinjected with 10 mM (1,3,4,5)IP\textsubscript{4} (indicated by the arrow on the left), and the horizontal bar indicates when extracellular \[Ca^{2+}\] was restored to 1.8 mM. This was followed, where indicated, by 2 μM thapsigargin.](https://example.com/fig1.png)

![Fig. 2. The effect of inositol polyphosphate microinjection on Ca\textsuperscript{2+} mobilization and entry in a mouse lacrimal acinar cell previously microinjected with a submaximal concentration of (2,4,5)IP\textsubscript{3}. As in Fig. 1, lacrimal cells were incubated in a nominally Ca\textsuperscript{2+}-free medium during the microinjections (indicated with arrows), while the horizontal bars indicate when extracellular \[Ca^{2+}\] was restored to 1.8 mM. a, (2,4,5)IP\textsubscript{3} was first injected as indicated by the first arrow. The pipette solution contained 1 mM (2,4,5)IP\textsubscript{3} which gave sufficient intracellular (2,4,5)IP\textsubscript{3} to induce a submaximal release of Ca\textsuperscript{2+}. The subsequent injection of submaximal (2,4,5)IP\textsubscript{3} (second arrow) caused a second, additional release of intracellular Ca\textsuperscript{2+}, and the cumulative effects of these injections resulted in an increased level of Ca\textsuperscript{2+} entry, b, as a control for any possible effect that the injection procedure itself may have on the Ca\textsuperscript{2+} entry phase itself, a second injection was made in the absence of any inositol phosphate. In c and d, the protocol was similar to that described for a, except that the second injection was either 100 μM (1,3,4,5)IP\textsubscript{4} (c) or 10 mM (1,3,4,5)IP\textsubscript{4} (d). In all cases, the injection did not induce intracellular Ca\textsuperscript{2+} mobilization, nor did it potentiate Ca\textsuperscript{2+} entry. However, with high concentrations of (1,3,4,5)IP\textsubscript{4}, the subsequent Ca\textsuperscript{2+} entry phase was reduced, and in some cases it was blocked. Each experiment is representative of three to seven observations.](https://example.com/fig2.png)
After establishing the response of a single lacrimal cell to a submaximal concentration of (2,4,5)IP₃, the cells were returned to a nominally Ca²⁺-free medium and microinjected a second time with different concentrations of (1,3,4,5)IP₄ (Fig. 2, c and d). In all cases, (1,3,4,5)IP₄ (pipette concentrations from 100 μM to 10 mM) neither released additional intracellular Ca²⁺ nor potentiated the Ca²⁺ entry phase seen with the (2,4,5)IP₃ alone (n = 15/15). Rather, high concentrations of microinjected (1,3,4,5)IP₄ appeared to reduce the subsequent Ca²⁺ entry phase, and the highest concentrations used almost completely blocked the entry phase (Fig. 2d, 10 mM (1,3,4,5)IP₄ in the pipette, cellular concentration 100–200 μM; n = 6/6). Note that although the Ca²⁺ entry phase appears blocked, Ca²⁺ entry can still be activated by treatment with thapsigargin (Fig. 1).

Fig. 3 summarizes results of experiments showing that the inhibition of calcium entry by injected (1,3,4,5)IP₄ was dependent on the concentration of (1,3,4,5)IP₄ in the injection pipette. We next considered that the inhibitory effect of (1,3,4,5)IP₄ might result from an inhibition of the action of (2,4,5)IP₃ at the IP₃ receptor. Thus, we examined the effect of (1,3,4,5)IP₄ on (2,4,5)IP₃ release from saponin-permeabilized mouse lacrimal cells, particularly to see if (1,3,4,5)IP₄ could modulate the effect of a submaximal concentration of (2,4,5)IP₃ on (2,4,5)IP₃ release (Fig. 4). In permeabilized lacrimal cells, (2,4,5)IP₃ released (2,4,5)IP₃ in a dose-dependent fashion (EC₅₀ ≈ 6.5 ± 0.8 μM; maximal release was 46.2 ± 4.8% of the ATP-dependent (2,4,5)IP₃ pool). As shown in Fig. 4, a submaximal concentration (10 μM) of (2,4,5)IP₃ released 31.8 ± 5.4% (n = 4) of the ATP-dependent (2,4,5)IP₃ pool (total ATP-dependent (2,4,5)IP₃ pool = 6.40 ± 0.98 nmol of Ca²⁺/mg of protein; n = 4). Under these conditions, (1,3,4,5)IP₄ (500 μM) did not cause significant (2,4,5)IP₃ release (4.0 ± 4.9% n = 4). When 10 μM (2,4,5)IP₃ and various concentrations of (1,3,4,5)IP₄ were added together to the permeabilized cells, (1,3,4,5)IP₄ did not augment Ca²⁺ release due to (2,4,5)IP₃. Rather, consistent with the observations in single cells, concentrations of (1,3,4,5)IP₄ greater than 10 μM apparently inhibited the ability of 10 μM (2,4,5)IP₃ to release (2,4,5)IP₃.

We also confirmed that (1,3,4,5)IP₄ was capable of inhibiting the Ca²⁺-mobilizing action of (2,4,5)IP₃ in intact cells. In experiments shown in Fig. 5, 10 mM (1,3,4,5)IP₄ was injected into a single lacrimal cell prior to injection of 1 mM (2,4,5)IP₃. This resulted in a complete blockade of both the Ca²⁺ release and Ca²⁺ entry phases of the response to (2,4,5)IP₃.

**DISCUSSION**

(1,3,4,5)IP₄ did not release intracellular Ca²⁺ in intact or permeabilized cells, nor did it induce or facilitate Ca²⁺ entry in intact cells. Rather, and surprisingly, (1,3,4,5)IP₄ appeared to block the Ca²⁺ entry phase induced by (2,4,5)IP₃ microinjection in intact cells. Results from experiments in permeabilized and intact cells would suggest that the inhibitory effect on the Ca²⁺ entry phase may be due to (1,3,4,5)IP₄ interfering with the ability of (2,4,5)IP₃ to maintain depletion of the intracellular Ca²⁺ pool. It is now well established that depletion of the
intrinsic Ca\textsuperscript{2+} pool by (1,4,5)IP\textsubscript{3} proportionally activates Ca\textsuperscript{2+} entry (17). Heparin, an antagonist of the (1,4,5)IP\textsubscript{3} receptor, blocks agonist-activated calcium entry presumably by virtue of its ability to prevent (1,4,5)IP\textsubscript{3}-induced depletion of intracellular stores (11); heparin does not block thapsigargin-activated calcium entry that does not involve interaction of (1,4,5)IP\textsubscript{3} with its receptor (11) (but see Ref. 18). (1,3,4,5)IP\textsubscript{4} similarly blocked calcium entry as well as calcium release by (2,4,5)IP\textsubscript{3} and the injected (1,3,4,5)IP\textsubscript{4}. Thus, although additional work is clearly needed, we suggest that inhibition by (1,4,5)IP\textsubscript{3} receptor to (1,4,5)IP\textsubscript{3} and/or to (1,3,4,5)IP\textsubscript{4} in intact cells may be markedly different than in permeable cells. Errors in estimates of cellular dilution of inositol phosphates are not an issue, because these would apply equally to the injected (1,4,5)IP\textsubscript{3} and the injected (1,3,4,5)IP\textsubscript{4}. Thus, although additional work is clearly needed, we suggest that inhibition by (1,3,4,5)IP\textsubscript{4} or possibly one of its metabolites, of the (1,4,5)IP\textsubscript{3} response may occur under conditions of physiological stimulation, and this could represent yet another important negative feedback on Ca\textsuperscript{2+} signaling, at least in this cell type.

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