

Different roles of IP₄ and IP₃ in the signal pathway coupled to the TRH receptor in microinjected *Xenopus* oocytes

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Different effects of injected IP₄ and IP₃ on membrane Cl⁻ currents were observed in *Xenopus* oocytes previously microinjected with GH₃ cell poly(A)⁺ RNA. IP₃ induced a biphasic current response similar to that induced by TRH, whereas IP₄ regularly evoked a monophasic current response consisting of a prolonged inward current with superimposed oscillations. Membrane currents elicited by TRH were enhanced by prior injection of IP₄, but not of IP₃. In contrast to IP₃-induced membrane currents, those evoked by IP₄ were independent of injection depth. Phorbol ester led to enhancement of the initial current after injection of IP₃, whereas the current oscillations were greater following injection of IP₄. The results indicate that, in *Xenopus* oocytes, IP₄ plays a role different from that of IP₃ within the signal transduction pathway initiated by ligand stimulation of a neuropeptide receptor.

Thyrotropin-releasing hormone; Hormone receptor; Chloride current; Voltage clamp; Inositol 1,4,5-trisphosphate; Inositol 1,3,4,5-tetrakisphosphate; (*Xenopus* oocyte)

1. INTRODUCTION

In a large variety of cells, neuropeptide-receptor binding activates a signal transduction pathway in which the formation of IP₃ plays an essential role [1,2]. IP₃ functions to release Ca²⁺ from intracellular stores leading, in *Xenopus* oocytes, to the activation of Ca²⁺-dependent plasma membrane Cl⁻ channels [3–5]. Recently, it has been proposed that IP₄, which is produced from IP₃ by phosphorylation [6], may also have a second messenger function in the control of Ca²⁺ metabolism [7]. Its mechanism of action, however, is still under discussion [2,8]. Whereas in sea urchin eggs a synergistic action of IP₄ and IP₃ in Ca²⁺ mobilization has been proposed [7], injection of IP₄ alone into *Xenopus* oocytes elicits Ca²⁺-dependent Cl⁻ currents [9]. In addition, a role for IP₄ in the sequestration of intracellular

Ca²⁺ has been reported [10]. To investigate the role of IP₄ in the intracellular signal transduction pathway, the effects of IP₄ and IP₃ on membrane currents in *Xenopus* oocytes were studied. The present data suggest that IP₃ and IP₄ have different functions in intracellular Ca²⁺ metabolism.

2. MATERIALS AND METHODS

Preparation of poly(A)⁺ RNA, injection of oocytes and voltage-clamp measurements of membrane currents were performed as in [11]. The Ringer's solution had the following composition (in mM): 116 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES-NaOH (pH 7.3). *D*-myo-Inositol 1,4,5-trisphosphate (IP₃) and inositol 1,3,4,5-tetrakisphosphate (IP₄) were purchased from Calbiochem, thyrotropin-releasing hormone (TRH), 4 β -phorbol 12-myristate 13-acetate (PMA) and 4 α -phorbol 12,13-didecanoate (PDD) being obtained from Sigma. IP₃ and IP₄ were dissolved in distilled water and stored as 1 mM stock solutions at -20°C. PMA and PDD were dissolved in dimethyl sulfoxide, stored as 4 mM stock solutions at -20°C and used at final concentrations of 10 and 100 nM, respectively. The temperature was 20–25°C.

Intracellular injections of IP₃ and IP₄ were made into the animal pole of the oocyte to avoid hemispheric differences [12]. For injection just below the plasma membrane, the pipette tip

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was pressed against the oocyte's surface until a shallow dimple appeared. The cell was then impaled by a slight tap on the head of the micromanipulator. For deep injections, microelectrodes were inserted about 200 μm into the oocyte [13]. The injection pipettes had tip diameters of 2–10 μm and were filled with 25 μM IP_3 or IP_4 . Micropipettes were calibrated to ensure the injection of defined liquid volumes. 5 nl were injected to give a final cytosolic concentration of 125 fmol IP_3 or IP_4 per oocyte.

3. RESULTS

TRH receptors were incorporated into the plasma membrane of *X. laevis* oocytes previously microinjected with poly(A)⁺ RNA from GH₃ [11,12]. Application of TRH to voltage-clamped oocytes evoked a biphasic membrane current consisting of a transient and a sustained inward current component with superimposed oscillations. Injection of IP_3 into oocytes evoked responses similar to those induced by receptor activation [5,14]. However, the shape of the IP_3 -evoked

response is largely dependent on the depth of the pipette tip within the oocyte [13]. As shown in fig.1, deep injections of IP_3 (15 oocytes/6 frogs) induced a slow inward current with large current fluctuations occurring after a long delay of 3–5 min. Under these conditions, the initial fast inward current was either absent or very small (fig.1A). Injection of IP_3 just below the plasma membrane (20 oocytes/7 frogs) evoked a rapid inward current of large amplitude, followed by a slow current which did not display significant current oscillations (fig.1B). In contrast, IP_4 -induced membrane currents did not depend on injection depth. Both deep (14 oocytes/6 frogs) and shallow (18 oocytes/7 frogs) injections of IP_4 induced slow inward currents with superimposed current oscillations (fig.1C,D). Fast transient current components did not occur. Fig.1 also shows that the time interval between injection and the onset of membrane current responses is considerably

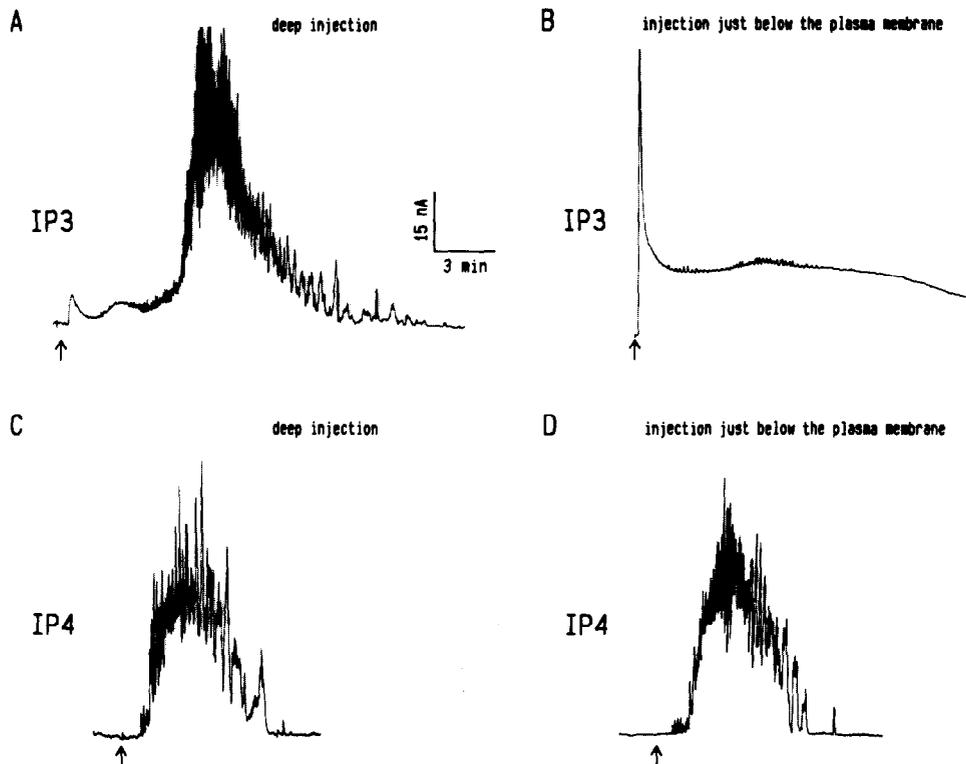


Fig.1. Injections of IP_3 (A,B) or IP_4 (C,D) (125 fmol) into four different *Xenopus laevis* oocytes. Injection of IP_3 deep into the cytoplasm (A) or just below the plasma membrane (B) elicited different membrane current responses. IP_4 -evoked responses were independent of injection depth (C,D). Oocytes were voltage-clamped at a holding potential of -60 mV. Upward deflection of membrane currents denotes inward current.

shorter for IP₄ injections than for deep injections of IP₃.

For an analysis of the effects of IP₃ and IP₄ upon the TRH-mediated current response, the ligand was applied to voltage-clamped oocytes, previously injected with IP₃ or IP₄. Injections of IP₃ had no effect on TRH-induced membrane responses (15 oocytes/4 frogs), whereas prior injections of IP₄ gave rise to an increase in subsequent TRH-generated membrane currents of approx. 2-fold (fig.2). The duration of the TRH-mediated responses as well as the frequency of the current oscillations did not change significantly. TRH-stimulated membrane currents were enhanced for 15 min after the end of the IP₄-generated current. Thereafter, the TRH-mediated membrane current response decreased to normal levels. As with prior injection of IP₄, the second oscillating part of the TRH response was enlarged when TRH application was preceded by injection of Ca²⁺ (not shown).

Recently, it has been shown that IP₃-induced membrane current responses are enhanced in oocytes following activation of protein kinase C by phorbol ester treatment [15–17]. Fig.3 demonstrates that under these conditions IP₄-induced membrane currents were also considerably greater (7 oocytes/3 frogs). Nevertheless, there were distinct differences between the effects of phorbol esters (10 nM PMA) on the responses evoked by IP₄ or IP₃. When PMA was present for 5 min, the IP₄-induced responses were characterized by the appearance of a small, rapid, initial cur-

rent, a 2–4-fold increase in amplitude of the slow inward current and enhancement of the current oscillations. This effect was still observed and in some oocytes even more pronounced when PMA treatment was extended to 35 min. The non-tumor-promoting α -derivative PDD (100 nM) had no effect. The IP₃-induced responses were also greater in the presence of PMA. However, this enhancement was restricted to a 5–8-fold increase in the fast, initial current component [15]. The slow inward current and the current oscillations remained almost unaffected by PMA.

4. DISCUSSION

The present data show that injection of IP₄ into *Xenopus* oocytes evokes membrane currents which exhibit distinct differences compared to those induced by IP₃. The enhancement in IP₄-induced current oscillations in the presence of tumor-promoting phorbol ester, as well as the increase in TRH-induced membrane currents brought about by prior injection of IP₄, indicate that IP₄ may predominantly be involved in the intracellular processes generating the sustained inward current and the superimposed current oscillations induced by application of TRH. IP₄ never elicited a rapid transient current which is characteristic for the first part of the neuropeptide-evoked current response and for the membrane currents elicited by injection of IP₃ just below the plasma membrane.

Our result showing that IP₄ injected into oocytes elicits Cl⁻ membrane currents supports a recently

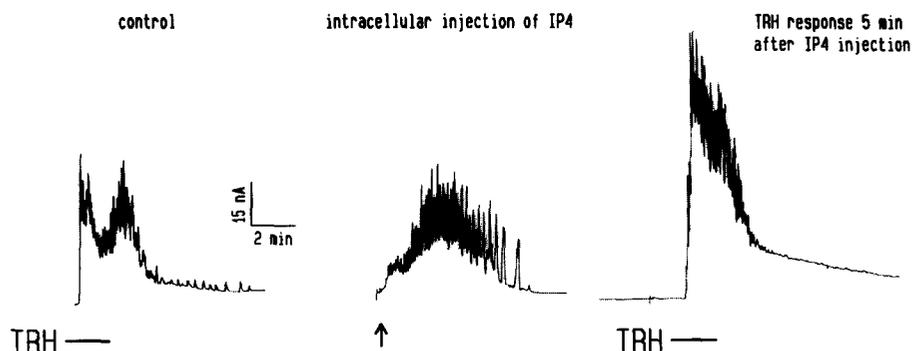


Fig.2. Enhancement of TRH-induced membrane current response by prior injection of IP₄. An oocyte, previously injected with GH₃ cell mRNA, was voltage-clamped and consecutively perfused with TRH (0.5 μ M, 2 min), injected with IP₄ (125 fmol), and again perfused with TRH (0.5 μ M, 2 min) 5 min after the end of the IP₄-induced response. Horizontal bars denote duration of TRH application. Scale bars denote 15 nA (vertical) and 2 min (horizontal).

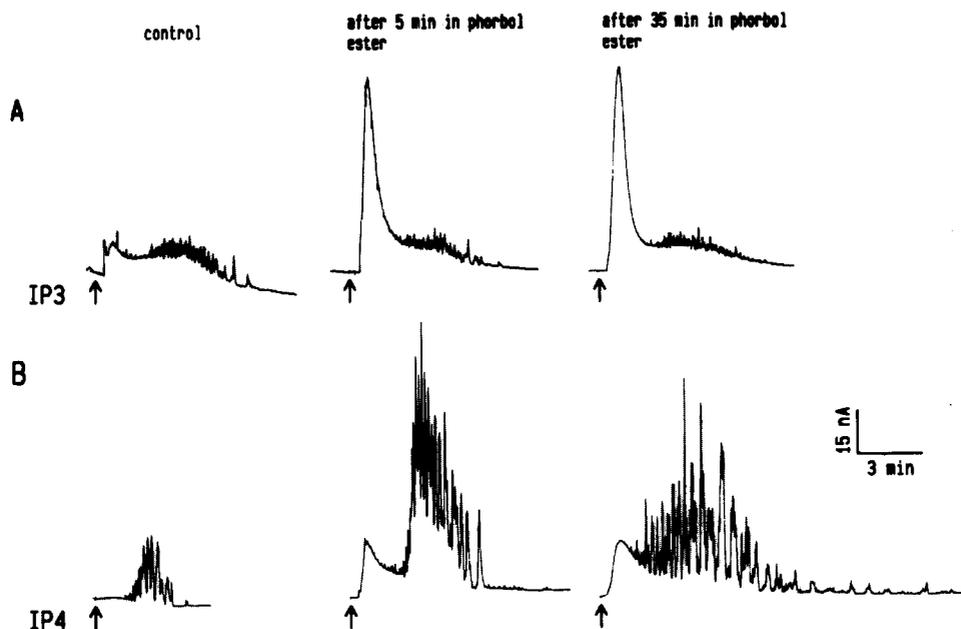


Fig.3. Different effects of PMA on IP₃- and IP₄-induced membrane current responses. Oocytes were voltage-clamped, incubated in 10 nM PMA, and injected with either IP₃ (A) or IP₄ (B), 5 or 35 min after the beginning of PMA incubation. Enhancement of membrane currents continued for at least 35 min.

reported observation by Parker and Miledi [9]. IP₄-induced currents are abolished by intracellular EGTA, but are not influenced by removal of Ca²⁺ from the external solution ([9]; and our unpublished data). This suggests that IP₄ leads to the release of Ca²⁺ from intracellular stores. In contrast to IP₃, which has been shown to cause immediate release of Ca²⁺ from IP₃-sensitive Ca²⁺ stores and to open Ca²⁺-dependent plasma membrane Cl⁻ channels, the direct release of Ca²⁺ from intracellular stores by IP₄ remains to be demonstrated [2,4,5,14]. Alternatively, the Ca²⁺ release after IP₄ injection could also be the result of an effect exerted by IP₃ generated from IP₄ by dephosphorylation [18].

The effects of IP₄ described here could indicate that IP₄ has a function in the slow oscillating part of the neuropeptide-induced membrane current response. In rat liver epithelial cells, IP₄ serves to sequester Ca²⁺ into intracellular stores [10]. IP₄ could also be involved in the control of Ca²⁺ channels connecting IP₃-sensitive and -insensitive intracellular Ca²⁺ pools [19]. It is conceivable that both of these possible functions of IP₄ are part of the complex intracellular processes underlying the

membrane current oscillations [20], although the exact role played by IP₄ within the signal transduction pathway remains to be determined.

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