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Different roles of IP_4 and IP_3 in the signal pathway coupled to the TRH receptor in microinjected *Xenopus* oocytes

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Different effects of injected IP_4 and IP_3 on membrane Cl^- currents were observed in *Xenopus* oocytes previously microinjected with GH_3 cell poly(A)$^+$ RNA. IP_3 induced a biphasic current response similar to that induced by TRH, whereas IP_4 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 IP_4, but not of IP_3. In contrast to IP_3-induced membrane currents, those evoked by IP_4 were independent of injection depth. Phorbol ester led to enhancement of the initial current after injection of IP_3, whereas the current oscillations were greater following injection of IP_4. The results indicate that, in *Xenopus* oocytes, IP_4 plays a role different from that of IP_3 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 IP_3 plays an essential role [1,2]. IP_3 functions to release Ca^{2+} from intracellular stores leading, in *Xenopus* oocytes, to the activation of Ca^{2+}-dependent plasma membrane Cl^- channels [3–5]. Recently, it has been proposed that IP_4, which is produced from IP_3 by phosphorylation [6], may also have a second messenger function in the control of Ca^{2+} metabolism [7]. Its mechanism of action, however, is still under discussion [2,8]. Whereas in sea urchin eggs a synergistic action of IP_4 and IP_3 in Ca^{2+} mobilization has been proposed [7], injection of IP_4 alone into *Xenopus* oocytes elicits Ca^{2+}-dependent Cl^- currents [9]. In addition, a role for IP_4 in the sequestration of intracellular Ca^{2+} has been reported [10]. To investigate the role of IP_4 in the intracellular signal transduction pathway, the effects of IP_4 and IP_3 on membrane currents in *Xenopus* oocytes were studied. The present data suggest that IP_3 and IP_4 have different functions in intracellular Ca^{2+} metabolism.

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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 $CaCl_2$, 1 $MgCl_2$, 10 Hepes-NaOH (pH 7.3). D-*myo*-Inositol 1,4,5-trisphosphate (IP_3) and inositol 1,3,4,5-tetrakisphosphate (IP_4) were purchased from Calbiochem, thyrotropin-releasing hormone (TRH), 4β-phorbol 12-myristate 13-acetate (PMA) and 4α-phorbol 12,13-didecanoate (PDD) being obtained from Sigma. IP_3 and IP_4 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 IP_3 and IP_4 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 GH_3 [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

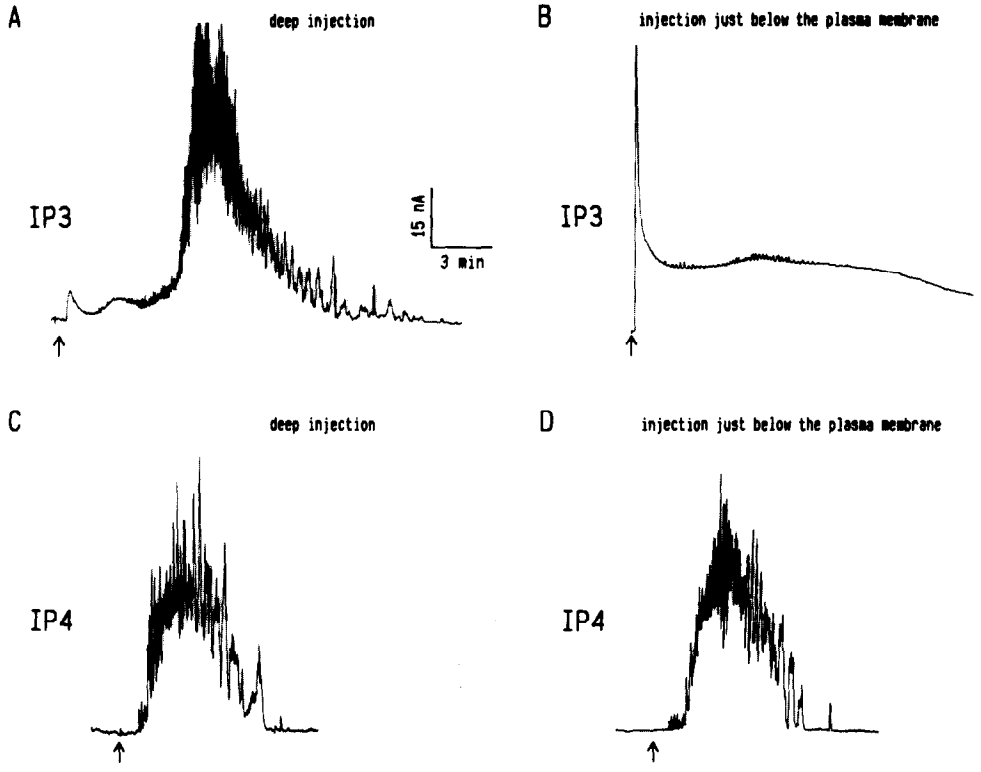

Fig.1. Injections of IP_3 (A,B) or IP_4 (C,D) (125 fmol) into four different *Xenopus laevis* oocytes. Injection of IP_3 deep into the cytoplasm (A) or just below the plasma membrane (B) elicited different membrane current responses. IP_4-evoked responses were independent of injection depth (C,D). Oocytes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

shorter for IP_4 injections than for deep injections of IP_3.

For an analysis of the effects of IP_3 and IP_4 upon the TRH-mediated current response, the ligand was applied to voltage-clamped oocytes, previously injected with IP_3 or IP_4. Injections of IP_3 had no effect on TRH-induced membrane responses (15 oocytes/4 frogs), whereas prior injections of IP_4 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 IP_4-generated current. Thereafter, the TRH-mediated membrane current response decreased to normal levels. As with prior injection of IP_4, the second oscillating part of the TRH response was enlarged when TRH application was preceded by injection of Ca^{2+} (not shown).

Recently, it has been shown that IP_3-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 IP_4-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 IP_4 or IP_3. When PMA was present for 5 min, the IP_4-induced responses were characterized by the appearance of a small, rapid, initial current, 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 IP_3-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 IP_4 into *Xenopus* oocytes evokes membrane currents which exhibit distinct differences compared to those induced by IP_3. The enhancement in IP_4-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 IP_4, indicate that IP_4 may predominantly be involved in the intracellular processes generating the sustained inward current and the superimposed current oscillations induced by application of TRH. IP_4 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 IP_3 just below the plasma membrane.

Our result showing that IP_4 injected into oocytes elicits Cl^- membrane currents supports a recently

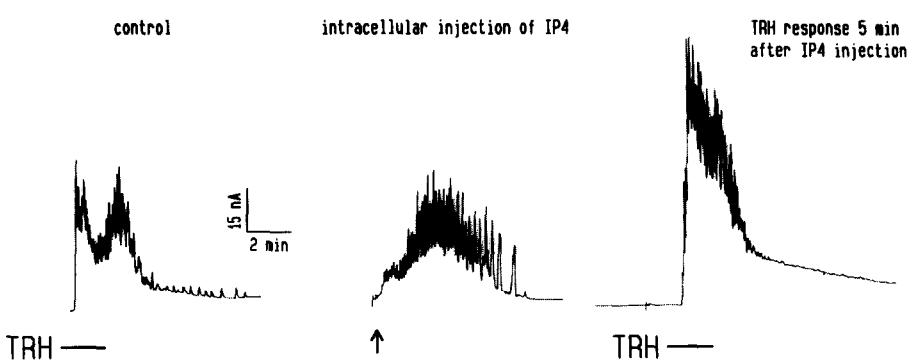

Fig.2. Enhancement of TRH-induced membrane current response by prior injection of IP_4. An oocyte, previously injected with GH_3 cell mRNA, was voltage-clamped and consecutively perfused with TRH (0.5 μM, 2 min), injected with IP_4 (125 fmol), and again perfused with TRH (0.5 μM, 2 min) 5 min after the end of the IP_4-induced response. Horizontal bars denote duration of TRH application. Scale bars denote 15 nA (vertical) and 2 min (horizontal).

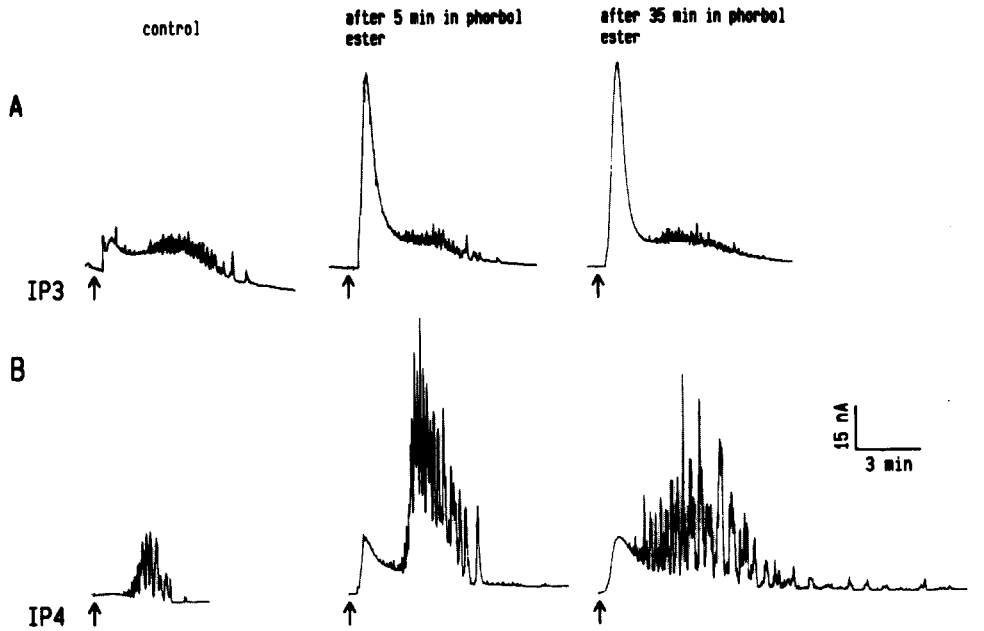

Fig.3. Different effects of PMA on IP_3- and IP_4-induced membrane current responses. Oocytes were voltage-clamped, incubated in 10 nM PMA, and injected with either IP_3 (A) or IP_4 (B), 5 or 35 min after the beginning of PMA incubation. Enhancement of membrane currents continued for at least 35 min.

reported observation by Parker and Miledi [9]. IP_4-induced currents are abolished by intracellular EGTA, but are not influenced by removal of Ca^{2+} from the external solution ([9]; and our unpublished data). This suggests that IP_4 leads to the release of Ca^{2+} from intracellular stores. In contrast to IP_3, which has been shown to cause immediate release of Ca^{2+} from IP_3-sensitive Ca^{2+} stores and to open Ca^{2+}-dependent plasma membrane Cl^- channels, the direct release of Ca^{2+} from intracellular stores by IP_4 remains to be demonstrated [2,4,5,14]. Alternatively, the Ca^{2+} release after IP_4 injection could also be the result of an effect exerted by IP_3 generated from IP_4 by dephosphorylation [18].

The effects of IP_4 described here could indicate that IP_4 has a function in the slow oscillating part of the neuropeptide-induced membrane current response. In rat liver epithelial cells, IP_4 serves to sequester Ca^{2+} into intracellular stores [10]. IP_4 could also be involved in the control of Ca^{2+} channels connecting IP_3-sensitive and -insensitive intracellular Ca^{2+} pools [19]. It is conceivable that both of these possible functions of IP_4 are part of the complex intracellular processes underlying the membrane current oscillations [20], although the exact role played by IP_4 within the signal transduction pathway remains to be determined.

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