

Membrane current responses to intracellular injections of inositol 1,3,4,5-tetrakisphosphate and inositol 1,3,4-trisphosphate in NG108-15 hybrid cells

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Iontophoretic injections of inositol 1,4,5-trisphosphate inside neuroblastoma × glioma NG108-15 hybrid cells evoked an outward K^+ current across the outer cell membrane, probably activated by the release of intracellular Ca^{2+} . No such current was produced by equivalent intracellular injections of inositol 1,3,4-trisphosphate or inositol 1,3,4,5-tetrakisphosphate. Instead, these compounds evoked an inward current with a reversal potential of about -20 mV, and which may therefore be due to a non-specific cation conductance. This suggests that these derivatives are unable to release sufficient Ca^{2+} to activate the Ca^{2+} -dependent K^+ current in these cells.

Inositol trisphosphate Inositol tetrakisphosphate Ca^{2+} release K^+ current

1. INTRODUCTION

Two novel inositol phosphates, inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate, have recently been described [1–3]. Irvine et al. [4] have shown that inositol 1,4,5-trisphosphate 3-kinase phosphorylates inositol 1,4,5-trisphosphate and produces inositol 1,3,4,5-tetrakisphosphate. The inositol tetrakisphosphate is dephosphorylated to inositol 1,3,4-trisphosphate [4]. In liver, in response to vasopressin, inositol 1,3,4,5-tetrakisphosphate increases at a slower rate than inositol 1,4,5-trisphosphate, but at a much faster rate than inositol 1,3,4-trisphosphate [5]. Similar responses have also been reported in pancreas cells [6] or neuroblastoma × glioma hybrid cells [7] in response to angiotensin and caerulein or bradykinin, respectively.

Inositol 1,4,5-trisphosphate stimulates release of stored Ca^{2+} into the cytoplasm [8]. Injections of inositol 1,4,5-trisphosphate into a neuroblastoma × glioma hybrid NG108-15 cell increase

cellular Ca^{2+} concentrations [9], and thereby generate a hyperpolarization (outward current) by activating a Ca^{2+} -dependent K^+ conductance [10,11]. In the present experiments we have used the presence of this Ca^{2+} -dependent K^+ current to test whether inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate can also release physiologically-significant amounts of Ca^{2+} when injected in NG108-15 cells.

2. MATERIALS AND METHODS

NG108-15 hybrid cells were cultured on polyornithine-coated dishes and differentiated with $10 \mu\text{M}$ prostaglandin E_1 and 1 mM theophylline, as described [10].

Inositol 1,4,5-trisphosphate was purchased from Calbiochem. Inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate were a generous gift from Dr Robin Irvine, Institute of Animal Physiology, Agricultural and Food Research Council, Cambridge, England. These three drugs were dissolved in distilled water at concentrations

of 0.5, 0.2 and 0.1 mM, respectively. The solutions were filled into micropipettes for iontophoretic injections. Intracellular injections into an NG108-15 cell were performed as described [10].

Membrane currents of the NG108-15 cells were measured with a second microelectrode filled with 1 M K citrate (20 M Ω) using a single electrode voltage-clamp method [11].

3. RESULTS AND DISCUSSION

Iontophoretic injections of inositol 1,4,5-trisphosphate into an NG108-15 cell voltage-clamped at -50 mV produced an outward membrane current accompanied by an increased input conductance (fig.1, left). This current is due to an increased K^+ conductance [11] and hence reverses to an inward current when the cell is hyperpolarized beyond E_K (about -80 mV: fig.2). Previous experiments have established that this current is a subspecies of Ca^{2+} -activated K^+ cur-

rent, which can also be activated by intracellular injections of Ca^{2+} and which is blocked by d-tubocurarine and apamin [11].

In contrast, equivalent injections of inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate evoked an inward current at -30 or -40 mV (fig.1, center and right), which could only be reversed to an outward current by depolarizing the cell beyond -20 mV (figs 1 and 2). This current is therefore not a K^+ current, but may be a non-specific cation current [12]. A similar current was sometimes evoked (in addition to the K^+ current) by large injections of inositol 1,4,5-trisphosphate.

A further distinction between inositol 1,4,5-trisphosphate and the other phosphates concerns the response to repeated injections (fig.3). The K^+ current induced by inositol 1,4,5-trisphosphate declined with repeated injection at intervals less than about 20 s. This may be due to depletion of the Ca^{2+} -storage sites [13]. In

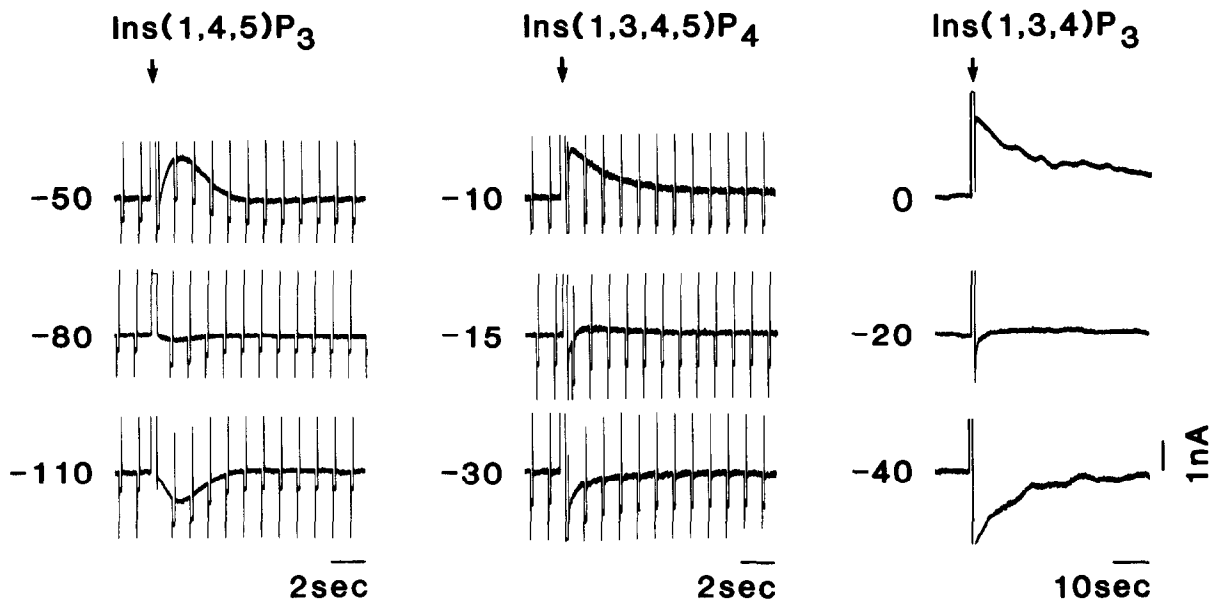


Fig.1. Membrane currents in an NG108-15 cell evoked by injections of inositol 1,4,5-trisphosphate [Ins(1,4,5)P₃], inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P₄] and inositol 1,3,4-trisphosphate [Ins(1,3,4)P₃]. The current was recorded at various holding potentials shown to the left of each example. Transient downward deflections are current-responses to constant hyperpolarizing voltage steps, used to measure cell input conductance. Injection currents (at arrows) were -55 nA \times 0.3 s for inositol 1,4,5-trisphosphate, -80 nA \times 0.3 s for inositol 1,3,4,5-tetrakisphosphate, and -150 nA \times 0.2 s for inositol 1,3,4-trisphosphate. Upward deflection indicates outward current.

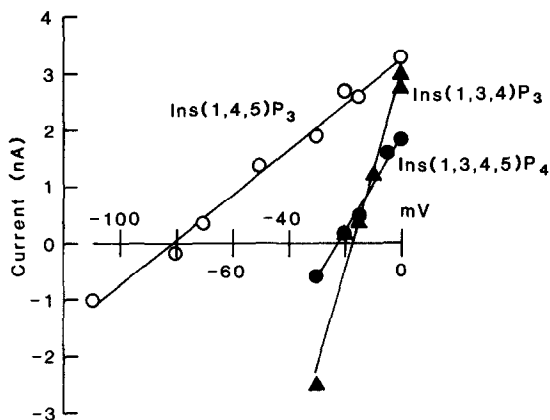


Fig.2. Plots of current amplitude (ordinates) in response to injections of inositol 1,4,5-trisphosphate (○), inositol 1,3,4,5-tetrakisphosphate (●) and inositol 1,3,4-trisphosphate (▲) as a function of holding potential (abscissa). Responses were obtained as shown in fig.1.

contrast, the equivalent current evoked by inositol 1,3,4,5-tetrakisphosphate did not show any decrease with repeated injections at 10 s intervals.

These results suggest either that any Ca^{2+} released by inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate activates a different membrane current to that released by inositol 1,4,5-trisphosphate or – more likely – that these compounds do not release appreciable Ca^{2+} when injected intracellularly, but instead generate an inward membrane current independent of Ca^{2+} release. The nature and significance of this current may warrant further investigation in view of the possible role of inositol phosphates as mediators of hormonal or transmitter actions.

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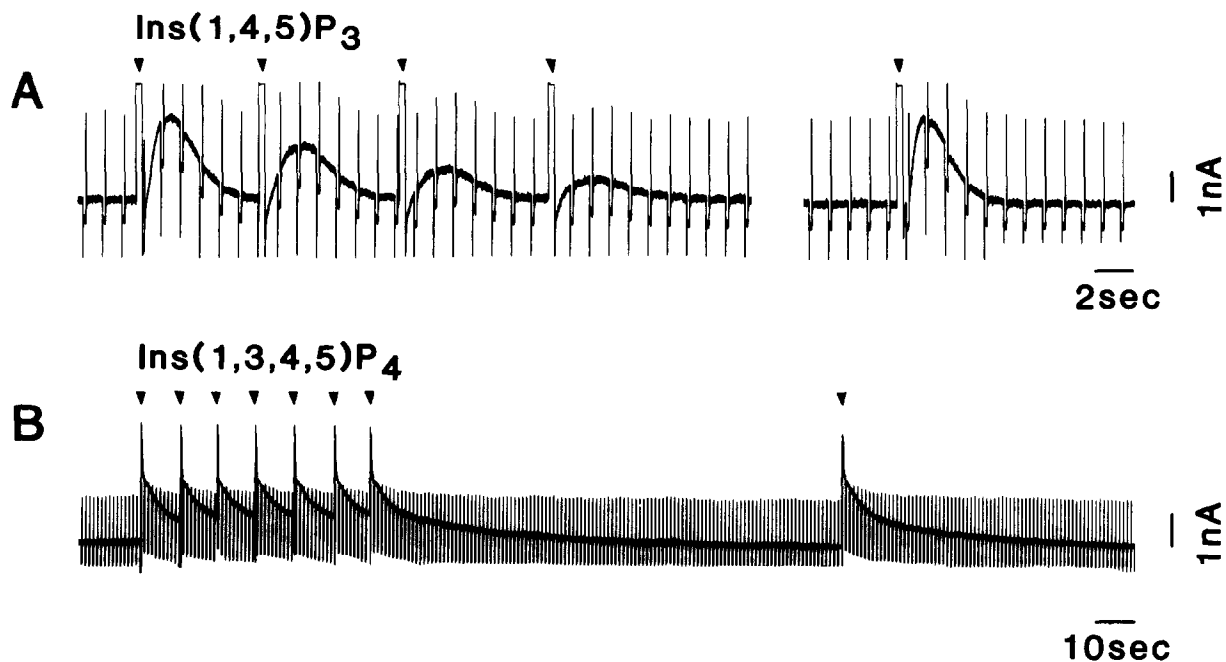


Fig.3. Effect of repeated injections of responses to inositol 1,4,5-trisphosphate (A) and inositol 1,3,4,5-tetrakisphosphate (B). Inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate were injected by a negative current of $-55 \text{ nA} \times 0.3 \text{ s}$ or $-35 \text{ nA} \times 0.2 \text{ s}$, respectively, at each arrowhead. A gap of 70 s elapsed between the fourth and fifth responses in A. Holding potential, -15 mV in A and 0 mV in B.

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