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 neuroblastsoma × 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 μM 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

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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 MΩ) 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 intput 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^+ current, 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

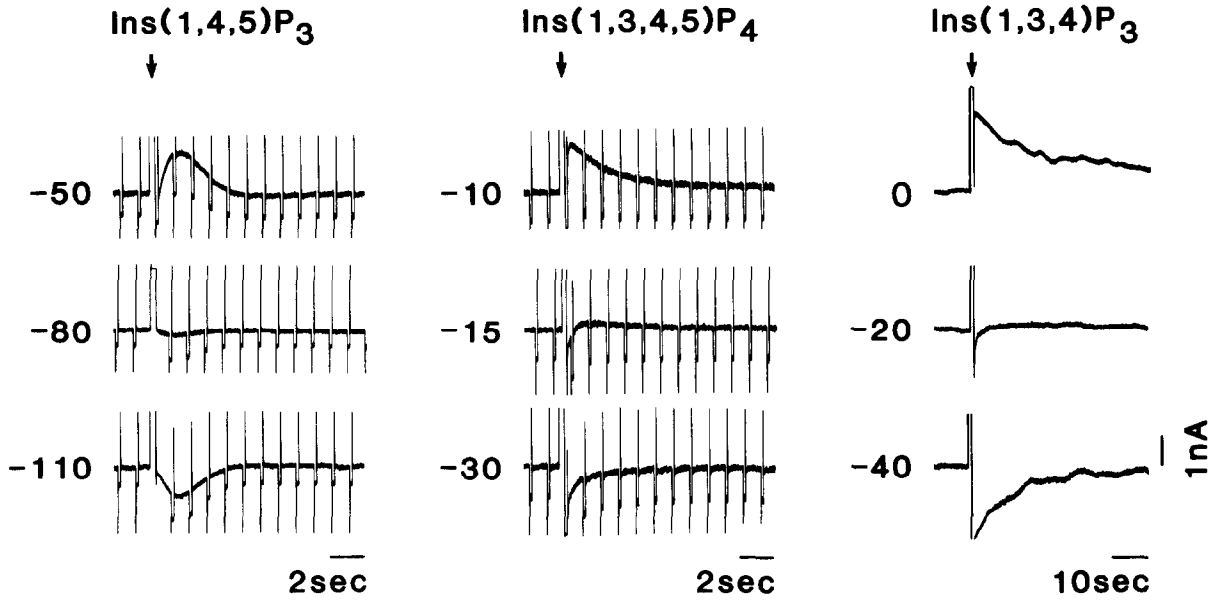

Fig.1. Membrane currents in an NG108-15 cell evoked by injections of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] and inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3]. The current was recorded at various holding potentials shown to the left of each example. Transient downward deflections are current-responses to constant hyperpolarizing voltage steps, used to measure cell input conductance. Injection currents (at arrows) were −55 nA × 0.3 s for inositol 1,4,5-trisphosphate, −80 nA × 0.3 s for inositol 1,3,4,5-tetrakisphosphate, and −150 nA × 0.2 s for inositol 1,3,4-trisphosphate. Upward deflection indicates outward current.

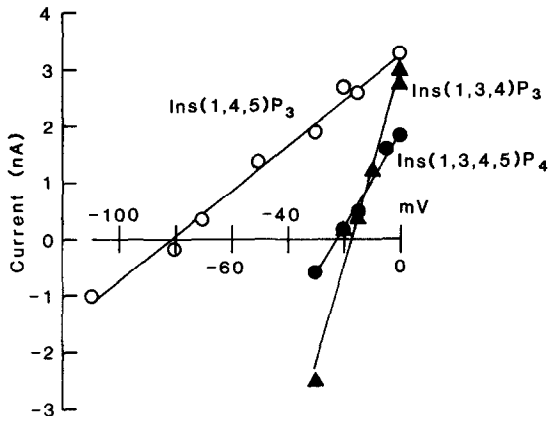

Fig.2. Plots of current amplitude (ordinates) in response to injections of inositol 1,4,5-trisphosphate (○), inositol 1,3,4,5-tetrakisphosphate (●) and inositol 1,3,4-trisphosphate (▲) as a function of holding potential (abscissa). Responses were obtained as shown in fig.1.

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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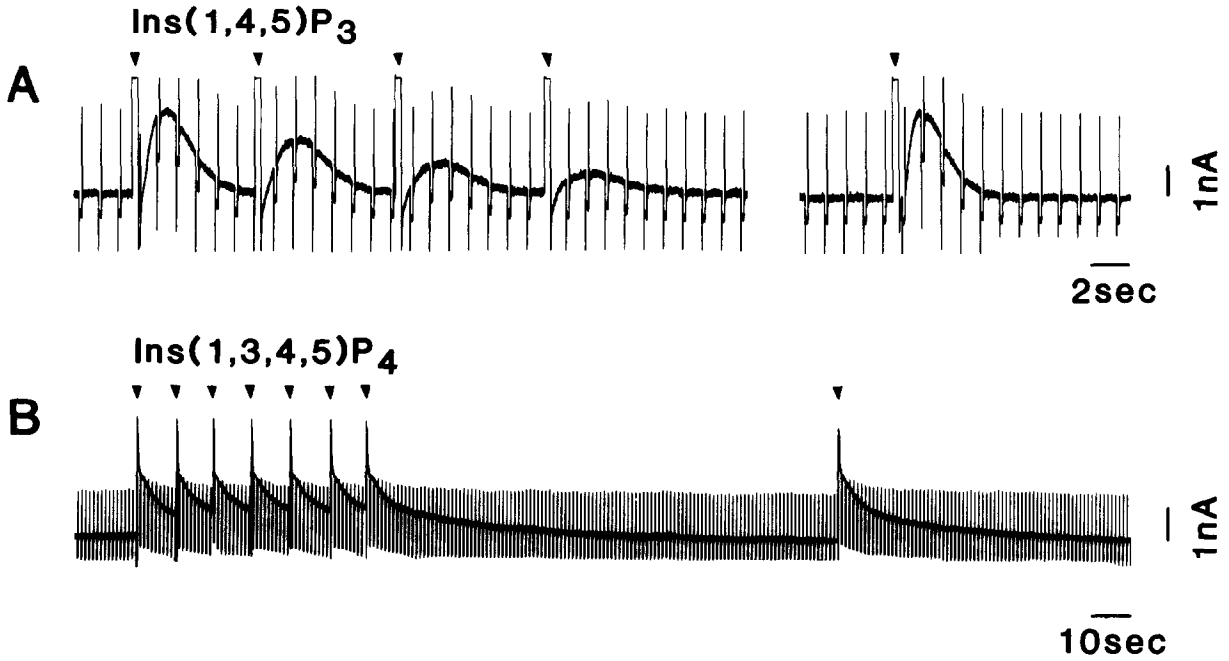

Fig.3. Effect of repeated injections of responses to inositol 1,4,5-trisphosphate (A) and inositol 1,3,4,5-tetrakisphosphate (B). Inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate were injected by a negative current of −55 nA × 0.3 s or −35 nA × 0.2 s, respectively, at each arrowhead. 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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