Volume 249, number 1, 105-107 FEB 07167 May 1989

Activation of chloride channels in the plasmalemma of *Nitella syncarpa* by inositol 1,4,5-trisphosphate

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Received 16 March 1989

The introduction of $1.5 \times 10^{-8} - 10^{-6}$ M inositol 1,4,5-trisphosphate (IP_3) into the bathing solution of *Nitella syncarpa* cells induced: (i) an increase in amplitude of the inward Ca^{2+} current; (ii) the appearance of an inward Cl^- current; (iii) a shift in the threshold for activation of the Cl^- channels on hyperpolarization of the membrane to more positive values. These facts suggest that IP_3 participates in the causing of changes in the intracellular free Ca^{2+} concentration and, hence, in the regulation of ionic channels in *Nitella syncarpa* plasmalemma.

Inositol 1,4,5-trisphosphate; Ca^{2+}; Ionic channel; (Characeae, *Nitella syncarpa*)

1. INTRODUCTION

In recent years the role of membrane phospholipid exchange in the regulation of intracellular Ca^{2+} has been widely studied [1,2]. Of particular interest in this regard is the exchange of minor membrane lipids, viz. phosphoinositides [3,4]. The products of inositol 1,4-bisphosphate (IP_2) hydrolysis, IP_3 and diacylglycerol, are secondary messengers [2]. Diacylglycerol triggers phosphorylation of regulatory proteins by activation of protein kinase C, whereas IP_3 participates in the release of Ca^{2+} from intracellular stores thus initiating a cascade of calcium-regulated reactions in the cell. In particular, intracellular calcium acts in the regulation of various types of ionic channels in membrane electrogenesis [5-7].

In membranes from a variety of plant cells, phospholipid exchange components and protein kinase C have been detected [8-10]. Moreover, IP_3 has been shown to be involved in the release of Ca^{2+} accumulated in protoplasts and microsomes [11,12].

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The present study was aimed at clarifying whether the product of phosphoinositide hydrolysis, IP_3, participates in controlling activation of ionic channels in the plasmalemma of *Nitella syncarpa*.

2. MATERIALS AND METHODS

Experiments were performed on perfused tonoplast-free cells of *N. syncarpa* under voltage-clamp conditions on the plasmalemma. The technique has been detailed in [14]. For intracellular and extracellular perfusion the following respective solutions (mM) were used: (i) 1 EGTA, 1.5 $MgCl_2$, 15 KCl, 10 Hepes-Tris, 0.5 ATP, 280 sucrose; $[Ca^{2+}]_{ext}$ 10^{-8} (pH 7.2); (ii) 1 $CaCl_2$; 5 Hepes-Tris, 220 sucrose, pH 7.2.

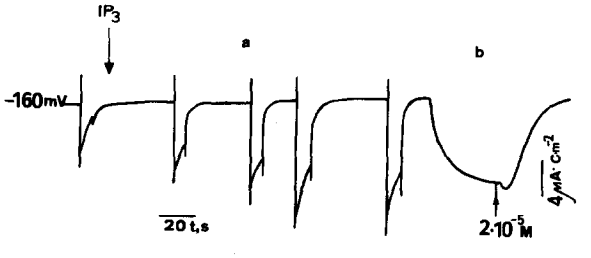

Fig.1. Increase in amplitude of the inward Ca^{2+} current at 50 mV (a) potential and development of the inward Cl^- current (b) in response to injection of 1.5×10^{-8} M IP_3 into the bathing solution. 2×10^{-5} M ethacrinic acid blocked the Cl^- current.

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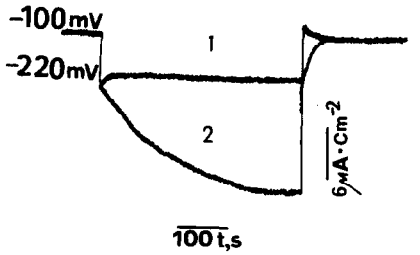

Fig.2. Shift of activation threshold for Cl^- channels on hyperpolarization under the influence of 1.5×10^{-8} M IP_3 introduced into the bathing solution. 1, control; 2, after addition of 1.5×10^{-8} M IP_3.

D-*myo*-Inositol 1,4,5-trisphosphate (Amersham) and ethacrynic acid (Sigma) were used.

3. RESULTS

Incubation of *Nitella* cells in an IP_3-containing solution led to an increase in amplitude of the Ca^{2+} current (I_{Ca}) developed on activation of the channels by depolarization. After a definite time period (10–20 min, depending on the IP_3 concentration), spontaneous generation of an inward current took place whose amplitude became larger with time. This current was easily blocked by ethacrynic acid, as reported for the case of *N. obtusa* cells [16]. We identified the current to be of chloride. Typical recordings of inward potential-dependent Ca^{2+} currents and Cl^- current are shown in fig.1.

The second set of experiments was carried out with Cl^- currents developed on shifting the fixed potential on the membrane toward hyperpolarization [15]. Under standard experimental conditions ($[Ca^{2+}]_{ext}$ 10^{-8} M) the threshold for Cl^- current generation was observed to be at -240 mV. When cells were incubated in an IP_3-containing solution, the treshold Cl^- current on hyperpolarization shifted markedly to positive values (fig.2).

4. DISCUSSION

As shown earlier [17], an increase in concentration of intracellular Ca^{2-} caused activation of potential-dependent Ca^{2+} channels in *N. obtusa* plasmalemma. The increase in I_{Ca} resulting from introduction of IP_3 into the bathing solution indicated that an increase occurred in intracellular Ca^{2+} level, most probably at the expense of the release from submembraneous stores.

Intracelluar Ca^{2+} also acts as a transmitter, capable of activating Cl^- channels in *N. obtusa* [7,14]. Ca^{2+} released from submembraneous stores and the local increase in Ca^{2+} concentration in the perimembrane layer might be the factors causing the development of a Cl^- current, as has been shown for oocytes [18].

The change in the threshold for activation of Cl^- channels of *Nitella* plasmalemma during hyperpolarization for incubation of cells in an IP_3 solution suggests that the Cl^- channels in *Nitella* cells are under the control of intracellular Ca^{2+}.

Thus, the data obtained on the ability of IP_3 to produce an increase in I_{Ca} and to induce the appearance of a Cl^- current through the *Nitella* plasmalemma, as well as its effect of shifting the threshold for activation of hyperpolarization-

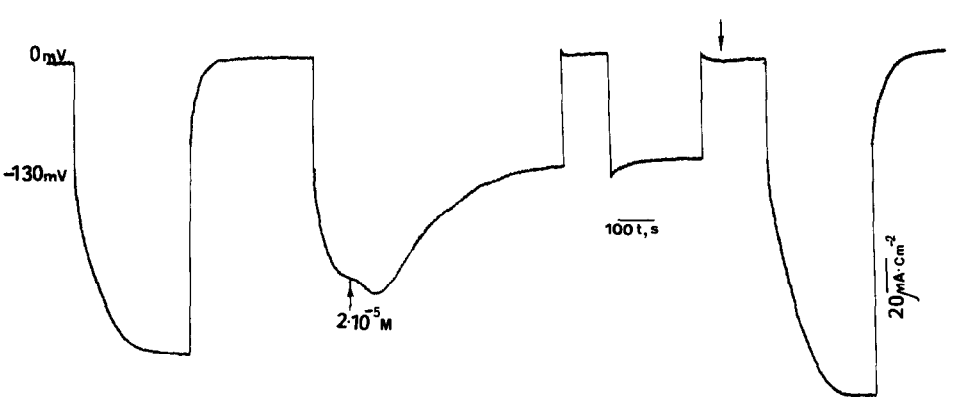

Fig.3. Reversible ethacrynic-acid-induced block of Cl^- currents on hyperpolarization (0 to -130 mV). The concentration of IP_3 was 10^{-6} M. ↑, addition of 2×10^{-5} M ethacrynic acid; ↓, washing from ethacrynic acid.

dependent Cl^- channels, constitute evidence in support of the contention that IP_3 promotes mobilization of intracellular Ca^{2+}.

The exchange of minor membrane lipids, namely phosphoinositides, probably plays an essential role in the regulation of ionic channels in the plasmalemma of Characeae.

Acknowledgements: The author expresses her gratitude to N. Agafonova for help in performing the present study and to M. Ilyina for technical assistance.

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