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Injected inositol 1,4,5-trisphosphate activates Ca^{2+}-sensitive K^+ channels in the plasmalemma of *Eremosphaera viridis*

Birgit Förster

Botanisches Institut I, Universität Würzburg, Würzburg, FRG

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$InsP_3$, an established mediator of intracellular Ca^{2+} signals in animal cells, is microinjected into the cytoplasm of *Eremosphaera viridis*. $InsP_3$, but not Ins, $InsP_1$, $InsP_2$ or F2,6-P_2 induce a transient opening of Ca^{2+}-dependent K^+ channels in the plasmalemma of this alga. This effect is indicated by a transient polarization (TP) with a simultaneous increase of membrane conductance. The TP is inhibited by TMB_8 (2 mM), an intracellular Ca^{2+} antagonist or by BAPTA (20 mM), microinjected together with $InsP_3$. The results suggest that $InsP_3$ initiates an increase in the cytoplasmic Ca^{2+} activity and an activation of Ca^{2+}-dependent membrane currents, hence, opening of K^+ channels.

$InsP_3$; K^+ channel, Ca^{2+}-dependent; Transient potential; *Eremosphaera viridis*

1. INTRODUCTION

In response to 'light off' and other external stimuli the unicellular green alga *Eremosphaera viridis* develops a rapid transient change of membrane potential (E_m) and conductance (g_m) (transient potential, TP) [1-3]. This TP is based on a transient and voltage-independent opening of K^+ channels in the plasmalemma. In the case of 'light off' induction the signal is passed on from the site of perception in the chloroplasts to the opening of K^+ channels in the plasma membrane. Recent data have established that an elevated cytosolic Ca^{2+} activity is of central significance for the signal transduction in *Eremosphaera* [4] and there is indirect evidence that also a mobilization of Ca^{2+} from intracellular stores (ER, vacuole) takes place [5,6].

The responses of mammalian cells to external stimuli are commonly mediated by the rapid turnover of plasma membrane-located phosphatidylinositol 4,5-bisphosphate with the production of the second messengers $InsP_3$ and diacylglycerol (DAG) [7-9]. While DAG is a natural activator of protein kinase C, $InsP_3$ can initiate Ca^{2+} release from nonmitochondrial intracellular stores into the cytoplasm [10]. Altered activities of cytosolic Ca^{2+} are known to trigger a variety of cell responses, ranging from activating a number of enzymes [11] to the regulation of membrane transport processes [10,12]. Many recent data support that this system occurs even in plants [13]. In plants, various transport mechanisms have been shown to be regulated by the cytoplasmic Ca^{2+} concentration [14-19].

Exogenously applied $InsP_3$ initiates Ca^{2+} efflux from vesicles derived from the tonoplast [20], isolated vacuoles [21] and isolated protoplasts [22]. Only few reports suggest involvement of $InsP_3$ in signal transduction in plants [23]. The present paper provides indirect evidence for the $InsP_3$-mediated Ca^{2+} release from internal stores and describes its activating effect on the Ca^{2+}-dependent K^+ channels in *Eremosphaera*.

Correspondence address: W. Simonis, Botanisches Institut I, Universität Würzburg, Mittlerer Dallenbergweg 64, 8700 Würzburg, FRG

Abbreviations: BAPTA, bis-(*o*-aminophenoxy)-ethane *N,N,N',N'*-tetraacetic acid; E_K, Nernst potential of potassium; E_m, membrane potential; ER, endoplasmic reticulum; F2,6-P_2, fructose 2,6-bisphosphate; Ins, myo-inositol; $InsP_1$, myo-inositol 2-monophosphate; $InsP_2$, myo-inositol 1,4-bisphosphate; $InsP_3$, myo-inositol 1,4,5-trisphosphate; TEA, tetraethylammonium; TMB_8, 3,4,5-trimethoxybenzoic acid 8-diethylaminooctyl ester; TP, transient potential

2. MATERIAL AND METHODS

2.1. *Culture conditions*

The coccal and unicellular green alga *Eremosphaera viridis* de Bary (Algal culture collection Göttingen LB 288-1, FRG) was maintained at 22°C in nutrient solution II as described by Köhler et al. [1].

2.2. *Chemicals*

Ins, $InsP_1$, $InsP_2$, $InsP_3$, F2, 6-P_2, TMB_8 and BAPTA were purchased from Sigma, other chemicals were from Fluka.

2.3. *Experimental procedure*

For electrophysiological measurements a spherical cell with an average diameter of 150 μm was selected for impalement. The external test medium contained (mM): 0.1 NaCl, 0.1 $MgCl_2$, 0.1 KNO_3, 0.1 $CaCl_2$. The pH was buffered at 5.6 with 2 mM MES/NaOH. The flow rate through the probe chamber was 120 ml/h.

Microinjections were made by means of the pressure probe [24]. The capillaries were filled by sucking up the solution, which was optically controlled. The tip diameter of the injection pipette, the pipette's diameter at the oil/solution boundary and the filling height

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were determined by means of an calibrated measuring ocular. From these data the volume of the injected solution could be calculated rather exactly. After placing the tip of a micropipette, filled with a definite quantity of solution, into the cytoplasm the pressure within the probe was increased to 2–3 bar. Subsequently, after healing-in of the glass capillaries, the external solution was adjusted to an osmolarity of about 340 mOsm by addition of sorbitol, so that the high turgor of the cell (9–11 bar) was reduced to 1–2 bar, allowing the solution in the micropipette to enter the cell. Neither electrical current nor pressure from the outside (because caused vibrations result in an TP) was applied. Under the conditions of change from high to low osmolarity the steady-state values of E_m are depolarized by about 10 mV.

2.4. *Experimental design*

Continuous measurements of both the transmembrane potential and the membrane resistance were carried out by the computer-supported single microelectrode technique. In order to determine the membrane and the electrode resistance, the computer generated bipolar pulses of 0.1 nA amplitude and 300 ms duration. Detailed description of the apparatus and methods has been given at Köhler et al. [2].

3. RESULTS AND DISCUSSION

As shown earlier an increase in the cytosolic Ca^{2+} activity leads to pronounced changes in the membrane transport properties of *Eremosphaera* due to the activation of K^+ channels in the plasmalemma [4,5]. To clarify the question whether this Ca^{2+} originated from the external medium via an entry through Ca^{2+} channels in the plasmalemma or from potential intracellular stores we investigated the activating effect of $InsP_3$ on the Ca^{2+}-sensitive K^+ channels in this alga.

The stimulating effect of $InsP_3$ microinjected into the cytoplasm of *Eremosphaera* is shown in Fig. 1. The injection pipettes with tip diameters of 3–4 μm were filled with 100 μM $InsP_3$ and were calibrated to ensure the injection of defined solution volumes. 0.02 nl were injected into an estimated cytoplasmic volume of 0.2–0.4 nl (10–20% of the total cell volume) to give a final cytosolic concentration of 5–10 μM $InsP_3$ (1–2 fmol per cell) (cf Table I), provided there is a uniform and rapid distribution of the injected compounds within the cytoplasm.

In the majority (>70%) of impaled cells of *Eremosphaera* $InsP_3$ injection evoked a large subsequent transient hyperpolarization of E_m within 20 s, with a slower relaxation back to the former steady state value. Corresponding to this TP a strong decline of membrane resistance could be observed. Compared to TPs triggered by 'light off' the duration of $InsP_3$-induced TPs is extremely prolonged from 37 ± 17 s ($n = 76$) to 378 ± 103 s ($n = 17$) (Table II). These TPs can be explained by an activation of Ca^{2+}-sensitive K^+ channels in the plasmalemma of *Eremosphaera*, giving rise to an increased outward K^+ current. The TP is characterized by a peak-point value in the range of E_K, comparable to the control-TP induced by 'light off', as shown ahead (Fig. 1). Moreover, TEA (10^{-2} mM) and Ba^{2+} (1 mM) suppressed these TPs by a direct block of K^+ channels (not shown).

Beside the long lasting TP a second pattern of responsiveness due to $InsP_3$ injection into *Eremosphaera* could be found (Fig. 2). Seven of twenty-four impaled cells generated oscillations of K^+ channel activation (repetitive TPs) immediately following injection of $InsP_3$. Attempts to induce oscillations by varying the $InsP_3$ concentration were unsuccessful.

$InsP_3$ appears to act in a very specific manner compared to other phosphorylated compounds or to non-substituted myo-inositol. In contrast to $InsP_3$ no TPs could be monitored in cells microinjected with Ins, $InsP_1$, $InsP_2$ or F2,6-P_2 (in 15–23 experiments each) (Table I). If $InsP_3$ was injected together with an excess of the Ca^{2+} chelator BAPTA (20 mM), $InsP_3$ failed to evoke any activating effect on the K^+ channels (not shown). On the other hand, removal of extracellular

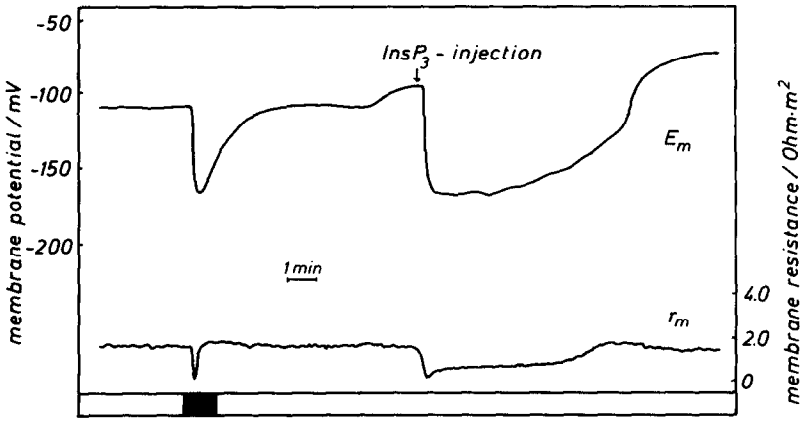

Fig. 1. Typical recording of membrane potential and resistance of *Eremosphaera* injected with $InsP_3$ (100 μM; $n = 17$). A control-TP, triggered by 'light off' is shown at the beginning of the trace. The light/dark program is represented as white and black bars. All curves are redrawn from original recordings. $[K^+]_o$ 0.1 mM, $[Ca^{2+}]_o$ 0.1 mM. E_m, membrane potential; r_m, membrane resistance.

Table I
Defined liquid volumes (0.02 nl) of the following compounds were microinjected into the cytoplasm of *Eremosphaera*

Compounds	Injected concentration (μM)	Estimated cytosolic concentration (μM)	TPs released
$InsP_3$	100	5–10	+ + +
$InsP_2$	100	5–10	+ – –
$InsP_1$	100	5–10	– – –
Ins	100	5–10	– – –
F2,6-P_2	100	5–10	– – –
TMB_8	$2 \cdot 10^3$	100–200	– – –
BAPTA	$2 \cdot 10^4$	10^3–$2 \cdot 10^3$	– – –
Ca^{2+}	10^3	50–100*	+ + +

* Since there is a certain ability of plant cells to buffer a rise in internal Ca^{2+}, the final cytosolic Ca^{2+} activity is much lower than calculated.

Table II
Characterization of TPs triggered by 'light off' and microinjected $InsP_3$ respectively microinjected Ca^{2+}. Number of experiments in brackets

Stimulus	'Light off' (76)	$InsP_3$ (17)	Ca^{2+} (14)
Released TPs (%)	53	88	93
Duration of TP (s)	38 ± 18	379 ± 104	197 ± 75
Duration of plateau phase (s)	9 ± 3	213 ± 97	101 ± 45

Ca^{2+} had no effect on the $InsP_3$-mediated channel activation.

The injection of a high concentration of Ca^{2+} into the cytoplasm could induce a response of similar magnitude to that induced by $InsP_3$, consisting of a prolonged activation of K^+ channels (Table II). A typical recording of a TP induced by internal Ca^{2+} application is shown in Fig. 3. This result is similar to those of rapidly increasing the internal Ca^{2+} activity by the ionophore A23187, which also evoked a long lasting TP in *Eremosphaera*, as previously demonstrated by Thaler et al. [4]. Oscillations of membrane potential and resistance could never be observed after Ca^{2+} injection. The Ca^{2+}-induced channel activation was subsequently abolished by TEA (10^{-2} mM) (not shown).

Microinjection of TMB_8 (Table I), known to be a Ca^{2+} antagonist, inhibited the $InsP_3$-induced effect in each of seven experiments (not shown), as well as the TPs triggered by 'light off'. TMB_8 has been shown to inhibit the Ca^{2+} release from vesicles derived from the tonoplast [20] and in vacuolar membranes it depressed an $InsP_3$-induced Ca^{2+} current [25], either by directly blocking of Ca^{2+} release or by interference with the interactions between $InsP_3$ and its receptor.

It has been firmly established in a wide variety of animal systems [review see 26] that $InsP_3$ mediates Ca^{2+} mobilization by directly opening Ca^{2+} channels, presumably in the ER. The information on the role of inositol phosphates in signal transduction within plants is steadily accumulating. In this context, the vacuole, able to accumulate 0.1–10 mM Ca^{2+} [27,28], has been found to be responsive to $InsP_3$. Several results have demonstrated the release of Ca^{2+} by $InsP_3$ from protoplasts [22], microsomal fractions [29–30], vacuolar membrane vesicles [20] or intact vacuoles [21]. From experiments using the patch clamp technique it could be shown that the tonoplast contains $InsP_3$-sensitive Ca^{2+} channels [25].

In view of the well established messenger function of $InsP_3$, the data obtained on the ability of $InsP_3$ to trigger TPs in *Eremosphaera* support the idea that $InsP_3$ may be involved in the regulation of Ca^{2+}-dependent K^+ channels in the plasmalemma of this alga. Since the $InsP_3$ response is blocked by Ca^{2+} chelation and can be mimicked by internal Ca^{2+} application, the simplest interpretation is that the effect is primarily caused by an increase in internal Ca^{2+} concentration evoked by $InsP_3$-mediated Ca^{2+} release. We found the long lasting

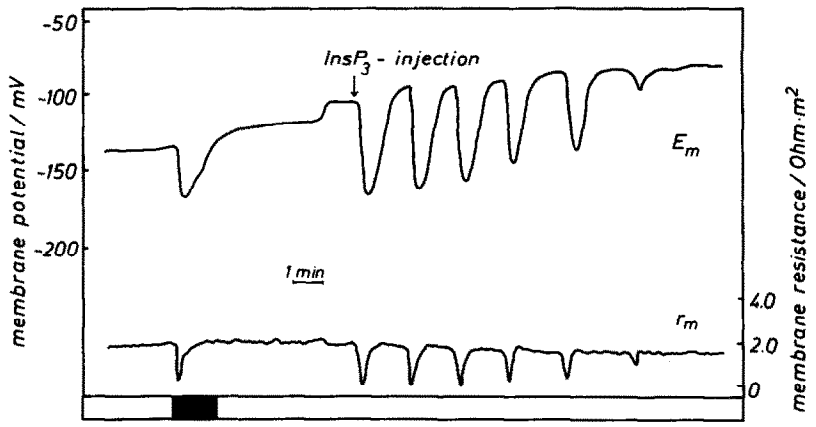

Fig. 2. Representative recording illustrating the oscillating response of *Eremosphaera* plasmalemma to microinjected $InsP_3$ ($n = 7$). Before $InsP_3$ was injected a TP was released by 'light off' to test the excitability of the cell. Further details see Fig. 1.

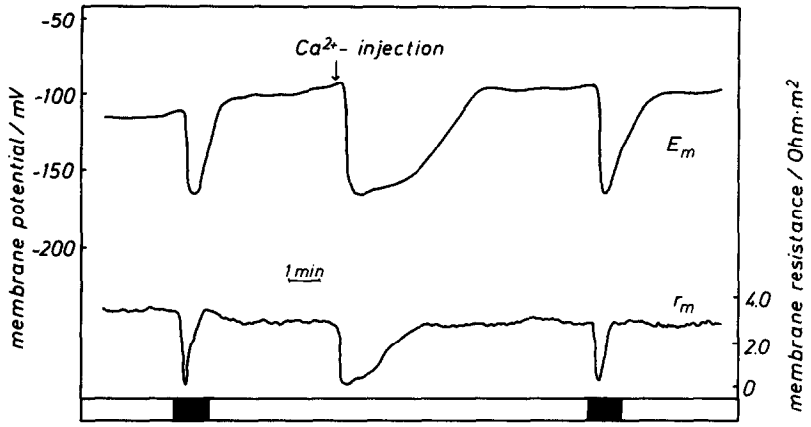

Fig. 3. The effect of injecting Ca^{2+} into the cytoplasm of *Eremosphaera* compared to a control-TP, elicited by 'light off' ($n = 14$). Further details see Fig. 1.

TP in agreement with previous experiments, where comparable prolonged TPs could be evoked by the Ca^{2+} ionophore A23187 [4], implying a sustained increased cytoplasmic Ca^{2+} activity.

In a number of cells injection of $InsP_3$ induced oscillations of membrane potential and resistance similar to those which occur in response to caffeine [5,6], known to release Ca^{2+} from intracellular stores. Since the plasma membrane possesses Ca^{2+}-dependent K^+ channels it is suggested that oscillation in membrane potential reflects an underlying oscillation in cytosolic Ca^{2+}, dependent upon the periodic release of stored Ca^{2+}. Consistent with these results are the observations that $InsP_3$ induced oscillatory activity in a variety of animal cells [10,12].

Increases in cytosolic Ca^{2+} have been associated with changes in membrane conductance in other systems too. Injection of $InsP_3$ into the *Limulus* ventral photoreceptor increases intracellular free Ca^{2+} and the conductance of ionic channels [31]. In Xenopus oocytes $InsP_3$ functions to the activation of Ca^{2+}-dependent plasma membrane Cl^- channels [32]. Also for plant cells Zherelova [33] previously demonstrated the regulation of Ca^{2+}-sensitive Cl^- channels by $InsP_3$.

Indeed, $InsP_3$ injection mimicks the 'light off'-induced opening of K^+ channels in *Eremosphaera*. This may be an indication that polyphosphoinositols are involved in the signal transduction pathway in *Eremosphaera* cells. However, whether $InsP_3$ is produced during the signal transduction from 'light off' to channel activation in vivo remains still to be determined.

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