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Protein kinase C is involved in regulation of Ca^{2+} channels in plasmalemma of *Nitella syncarpa*

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Ca^{2+} current recordings have been made on *Nitella syncarpa* cells using the intracellular perfusion and the voltage-clamp technique. TPA (12-*O*-tetradecanoylphorbol-13-acetate), a substance capable of activating protein kinase C from plasmalemma of *Nitella* cells, modulates voltage-dependent Ca^{2+} channels. Polymixin B, inhibitor of protein kinase C, blocks the *Nitella* plasmalemma Ca^{2+} channels; the rate of channel blockage depends on the concentration and exposure time of the substance.

Ca^{2+} channel; Protein kinase C; (*Nitella syncarpa*)

1. INTRODUCTION

It has been convincingly shown that the intracellular regulation of Ca^{2+} channels in the excitable animal cell membranes is mediated by phosphorylation of membraneous proteins by cAMP-dependent protein kinase [1–3]. The presence of Ca^{2+}-calmodulin-dependent protein kinase [4,5] and their participation in the phosphorylation of membraneous proteins have been also shown for plant tissues [6]. The functional state of Ca^{2+} channels of *Charophyta* plasmalemma has been shown to be controlled by a system of those enzymes regulating the cAMP level in the cell [7]. However, in the last few years another possible way of ionic channel regulation has been extensively studied associated with the exchange of the minor membraneous lipids, phosphoinositides [8,9]. The Ca^{2+} phospholipid-dependent enzyme, protein kinase C, detected by Nishizuki et al. is closely related with the phosphoinositide exchange [10]. Hydrolysis of one of these lipids, phosphatidylinositol-4,5-bisphosphate, leads to formation of two intermediates: inositol-1,4,5-trisphosphate and diacylglycerol, which act as secondary messengers [11]. Inositol-trisphosphate mobilizes Ca^{2+} from the intracellular stores [12,13], while diacylglycerol is a natural activator of protein kinase C. However, Nishizuki et al. [14] showed that a similar effect is also produced by the phorbol ester, a potent tumor promotor, for which protein kinase C is possibly a receptor. Presently phorbol esters such as TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) are used as selective activators of protein kinase C [9,15].

Protein kinase C has been identified in plant tissues and the first steps have been made in determining its functional role in the cell [16,17].

The objective of our study was to clarify whether protein kinase C is involved in regulation of voltage-dependent Ca^{2+} channels in the plasmalemma of *N. syncarpa* cells.

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2. MATERIALS AND METHODS

The study was performed on the plasmalemma of perfused *Nitella* cells by the voltage clamp technique. The protein kinase C activator was TPA (P.L. Biochemicals, USA) at concentrations from 10^{-9} to 10^{-11}; the inhibitor was the polypeptide antibiotic polymyxin B from Serva. The solutions used for the intracellular and external perfusion are presented in the figure

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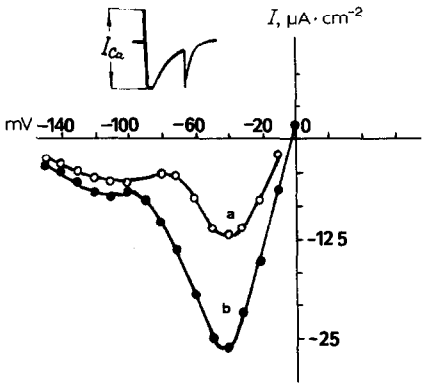

Fig.1. Activation of plasmalemma Ca^{2+} channels by intracellularly introduced Mg^{2+} and ATP. The current-voltage characteristics of Ca^{2+} channels registered by the pulse technique by changing the clamped potential from −150 mV to zero (each point corresponds to an I_{Ca} value differing by 10 mV compared to neighbouring ones). (a) Under perfusion with the following solution: 1.0 mM EGTA; 1.5 mM $MgCl_2$; 15 mM KCl, 280 mM sucrose, 5 mM Hepes-Tris (pH 7.2), 3–5 × 10^{-8} M $[Ca^{2+}]_{ins}$; (b) under perfusion with the same solution but with addition of 0.50 mM ATP. The external solution contained: 220 mM sucrose; 1 mM Hepes-Tris (pH 7.2), 1 mM $CaCl_2$.

legends. The Ca^{2+} channels of *N. syncarpa* plasmalemma, like those of *Nitellopsis* plasmalemma [18], are sensitive to the presence of Mg^{2+} and ATP in the intracellular solution (fig.1). However, the kinetics of activation and inactivation of Ca^{2+} channels in these cells is 2 to 3 times higher than in *Nitellopsis*. Their current-voltage characteristics under standard conditions have two pronounced maxima, which points to at least two Ca^{2+} channel populations in the plasmalemma. Animal cells are known at present to have three Ca^{2+} channel populations which are distinguished by different levels of activation potentials [19]. The activation threshold of *Nitella* Ca^{2+} channels is in the range of high negative potentials (at a fixation potential of −180 mV the activation threshold is −175 mV). Ca^{2+} channels of *Nitella* are more stable to perfusion than those of *Nitellopsis*. $K_{1/2}$ for nitrendipine, a blocker of Ca^{2+} channels, is nearly 2 × 10^{-6} M. The well known inhibitor of calmodulin, R24571, at a concentration of 10^{-7} M and lower activates Ca^{2+} channels in plasmalemma and the current-voltage characteristics show a specific third maximum which is probably due to the activation of the third Ca^{2+} channel population. Increase in the R24571 concentration up to 10^{-5} M produces a blocking of the electrogenic component and the Ca^{2+} current amplitude (I_{Ca}). The given characteristics of Ca^{2+} channels in *Nitella* plasmalemma are similar to those of Ca^{2+} channels in other objects.

3. RESULTS AND DISCUSSION

TPA (10^{-9}–10^{-11} M) introduced to the external solution markedly changed the activation of plasmalemma Ca^{2+} channels. Ca^{2+} currents predisposed to degradation during perfusion remained unchanged for a long time (above 2 h) when in solution with TPA. In 6 of 16 cases not only stabilization but also increase of I_{Ca} was observed (fig.2). However, it should be noted that in 5 cases I_{Ca} was observed to fall on exposure of the cell to TPA. TPA also induces the appearance of an outward current which corresponds to hyperpolarization, i.e. the electrogenic component is activated. The current density increased at an average by 0.015 mcA/cm^2 within 15 to 17 min.

The polypeptide antibiotic polymixin B, an inhibitor of phospholipid-sensitive Ca^{2+}-dependent protein kinase (protein kinase C) [19] when introduced to the external solution, always led to a block of I_{Ca} (fig.3). The blockage degree depended on the concentration of the drug and on the time of its action on the plasmalemma.

Thus the evidence obtained suggests that protein kinase C is involved in activation of Ca^{2+} channels and of the electrogenic component. This may be due to the phosphorylation of membraneous proteins by this enzyme.

Probably, the increase in the Ca^{2+} current amplitude on exposure to TPA is connected with activation of additional, 'silent' channels, as in the case of *Aplysia* neurons [20], or with the prolongation of the open state of the channel caused by

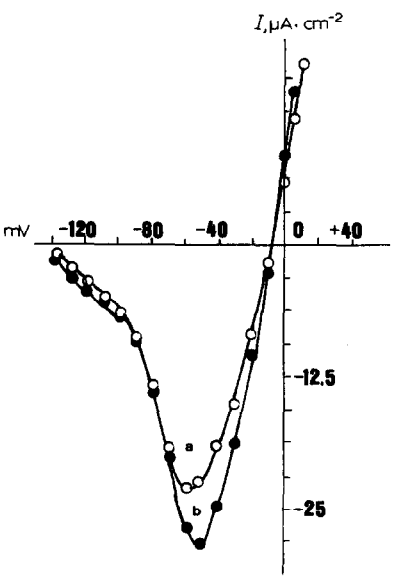

Fig.2. Effect of 10^{-11} M TPA introduced to the external solution on the amplitude of Ca^{2+} current. The solutions for perfusion are the same as described for fig.1.

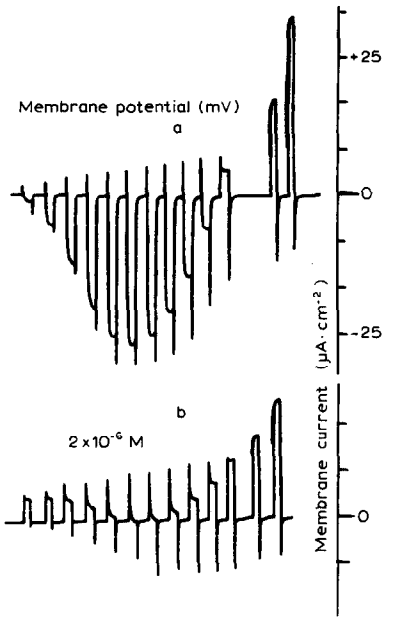

Fig.3. Block of I_{Ca} of plasmalemma by 2×10^{-6} M polymixin B, introduced to the external solution. The figure shows an original record of transient currents through the plasmalemma Ca^{2+} channels induced by a pulsed change of the fixed potential, V_m, from -100 mV to $+20$ mV. (a) Control; (b) effect of 2×10^{-6} M polymixin B.

slow dephosphorylation of the membraneous proteins involved in the Ca^{2+} channel structure. The appearance of the outward current corresponding to the electrogenic component seems to be due to activation of the plasmalemma H^+-ATPase [21]. This assumption is supported by the fact that inhibition of H^+-ATPase by vanadate suppressed the development of this component. The observed decrease in I_{Ca} by TPA is possibly due to the fact that the development of the hyperpolarization response inactivates the gating mechanism of Ca^{2+} channels, thus limiting the Ca^{2+} influx. This can be an indirect way for Ca^{2+} ions to regulate protein kinase C through the H^+-ATPase activity [22]. Another explanation for the I_{Ca} reduction could be the deficiency of lipids or of protein kinase C, itself in the membrane. Usually protein kinase C is reversibly activated and inactivated in the presence of Ca^{2+} and membraneous phospholipids [10].

The block of I_{Ca} by inhibition of protein kinase C by polymixin B also supports our assumption that protein kinase C participates in regulation of the activity of Ca^{2+} channels of plasmalemma of *Nitella* cells. It should be noted that the phosphorylation of membraneous proteins and variations in the intracellular Ca^{2+} concentration may be also involved in the mechanism of Cl^- channel activation in plant cell plasmalemma.

It would be of interest to study the role of protein kinase C and Ca^{2+} channel activation in the hormonal regulation of cell functions.

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REFERENCES

[1] Kostyuk, P.G., Doroshenko, P.A. and Martynyuk, A.E. (1984) Biol. Membrany 1, 18–26.
[2] Levitan, I.B. (1986) J. Membrane Biol. 87, 177–190.
[3] Curtis, B.M. and Cattarell, W.A. (1985) Proc. Natl. Acad. Sci. USA 82, 2528–2532.
[4] Dieter, P. (1984) Plant, Cell Environ. 7, 371–380.
[5] Salimath, B.P. and Marmé, D. (1983) Planta 158, 560–568.
[6] Veluthambi, K. and Poovaiah, B.W. (1984) Science 223, 167–168.
[7] Zherelova, O.M. and Grishenko, V.M. (1987) Dokl. AN SSSR 293, 1514–1516.
[8] Michell, R.H. (1975) Biochim. Biophys. Acta 415, 81–147.
[9] DeRiemer, S.A., Strong, J.A., Albert, K.A., Greengard, P. and Kaczmarek, L.K. (1985) Nature 313, 313–316.
[10] Nishizuka, Y., Takai, Y., Hashimoto, A., Kuroda, Y., Sakai, K. and Yamamura, H. (1979) Mol. Cell. Biochem. 23, 153–164.
[11] Berridge, M.J. and Irvin, R.F. (1984) Nature 312, 315–321.
[12] Eichberg, J. and Berti-Mattera, L.M. (1986) Prog. Brain Res. 69, 15–28.
[13] Schumaker, K.S. and Sze, H. (1987) J. Biol. Chem. 262, 3944–3946.
[14] Nishizuka, Y. (1984) Nature 308, 693–698.
[15] Doroshenko, P.A. and Kostyuk, P.G. (1987) Biol. Membrany 4, 1160–1163.
[16] Schäfer, A., Bygrave, F., Matzenauer, S. and Marmé, D. (1985) FEBS Lett. 187, 25–28.
[17] Selivankin, S.Yu., Romanenko, E.G., Novikova, G.V., Muromtseva, D.G. and Kulaeva, O.N. (1988) Fiziol. Rast. 35, 266–274.
[18] Zherelova, O.M., Kataev, A.A. and Berestovsky, G.N. (1985) Dokl. AN SSSR 281, 183–186.
[19] Mazzei, G.J., Katoh, N. and Kuo, J.F. (1982) Biochem. Biophys. Res. Commun. 109, 1129–1133.
[20] Strong, I.A., Fox, A.P., Tsien, R.W. and Kaczmarek, L.K. (1987) Nature 325, 714–717.
[21] Kasamo, K. and Nouchi, I. (1987) Plant Physiol. 83, 323–328.
[22] Zocchi, G., Rogers, S.A. and Hanson, J.B. (1985) Plant Sci. Lett. 40, 153–159.