

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

O.M. Zherelova

*Institute of Biological Physics, Acad. Sci. USSR, Pushchino, Moscow Region 142292, USSR*

Received 17 October 1988

$\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channels. Polymyxin B, inhibitor of protein kinase C, blocks the *Nitella* plasmalemma  $\text{Ca}^{2+}$  channels; the rate of channel blockage depends on the concentration and exposure time of the substance.

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

## 1. INTRODUCTION

It has been convincingly shown that the intracellular regulation of  $\text{Ca}^{2+}$  channels in the excitable animal cell membranes is mediated by phosphorylation of membraneous proteins by cAMP-dependent protein kinase [1-3]. The presence of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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-bisphos-

phate, leads to formation of two intermediates: inositol-1,4,5-trisphosphate and diacylglycerol, which act as secondary messengers [11]. Inositol-trisphosphate mobilizes  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channels in the plasmalemma of *N. syncarpa* cells.

## 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

*Correspondence address:* O.M. Zherelova, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, Moscow Region 142292, USSR

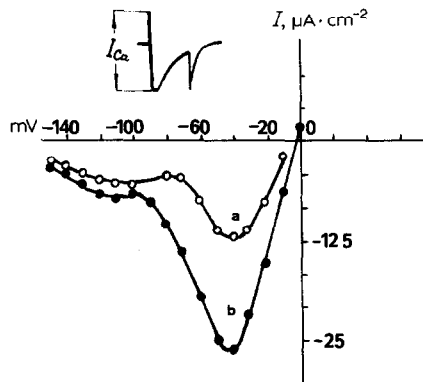


Fig.1. Activation of plasmalemma  $\text{Ca}^{2+}$  channels by intracellularly introduced  $\text{Mg}^{2+}$  and ATP. The current-voltage characteristics of  $\text{Ca}^{2+}$  channels registered by the pulse technique by changing the clamped potential from  $-150$  mV to zero (each point corresponds to an  $I_{\text{Ca}}$  value differing by  $10$  mV compared to neighbouring ones). (a) Under perfusion with the following solution:  $1.0$  mM EGTA;  $1.5$  mM  $\text{MgCl}_2$ ;  $15$  mM KCl,  $280$  mM sucrose,  $5$  mM Hepes-Tris (pH 7.2),  $3-5 \times 10^{-8}$  M  $[\text{Ca}^{2+}]_{\text{ins}}$ ; (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  $\text{CaCl}_2$ .

legends. The  $\text{Ca}^{2+}$  channels of *N. syncarpa* plasmalemma, like those of *Nitellopsis* plasmalemma [18], are sensitive to the presence of  $\text{Mg}^{2+}$  and ATP in the intracellular solution (fig.1). However, the kinetics of activation and inactivation of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channel populations in the plasmalemma. Animal cells are known at present to have three  $\text{Ca}^{2+}$  channel populations which are distinguished by different levels of activation potentials [19]. The activation threshold of *Nitella*  $\text{Ca}^{2+}$  channels is in the range of high negative potentials (at a fixation potential of  $-180$  mV the activation threshold is  $-175$  mV).  $\text{Ca}^{2+}$  channels of *Nitella* are more stable to perfusion than those of *Nitellopsis*.  $K_{1/2}$  for nitrendipine, a blocker of  $\text{Ca}^{2+}$  channels, is nearly  $2 \times 10^{-6}$  M. The well known inhibitor of calmodulin, R24571, at a concentration of  $10^{-7}$  M and lower activates  $\text{Ca}^{2+}$  channels in plasmalemma and the current-voltage characteristics show a specific third maximum which is probably due to the activation of the third  $\text{Ca}^{2+}$  channel population. Increase in the R24571 concentration up to  $10^{-5}$  M produces a blocking of the electrogenic component and the  $\text{Ca}^{2+}$  current amplitude ( $I_{\text{Ca}}$ ). The given characteristics of  $\text{Ca}^{2+}$  channels in *Nitella* plasmalemma are similar to those of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channels.  $\text{Ca}^{2+}$  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  $I_{\text{Ca}}$  was observed (fig.2). However, it should be noted that in 5 cases  $I_{\text{Ca}}$  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  $0.015$   $\mu\text{A}/\text{cm}^2$  within 15 to 17 min.

The polypeptide antibiotic polymyxin B, an inhibitor of phospholipid-sensitive  $\text{Ca}^{2+}$ -dependent protein kinase (protein kinase C) [19] when introduced to the external solution, always led to a block of  $I_{\text{Ca}}$  (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  $\text{Ca}^{2+}$  channels and of the electrogenic component. This may be due to the phosphorylation of membraneous proteins by this enzyme.

Probably, the increase in the  $\text{Ca}^{2+}$  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

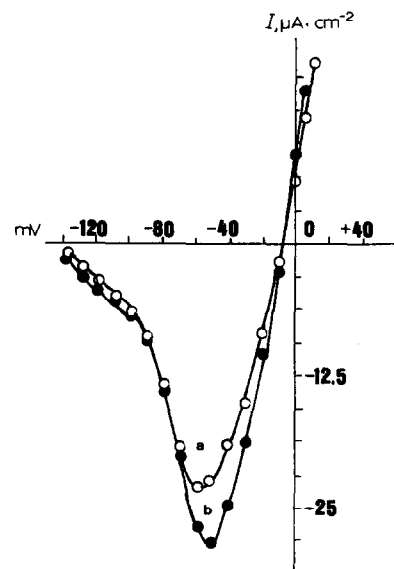


Fig.2. Effect of  $10^{-11}$  M TPA introduced to the external solution on the amplitude of  $\text{Ca}^{2+}$  current. The solutions for perfusion are the same as described for fig.1.

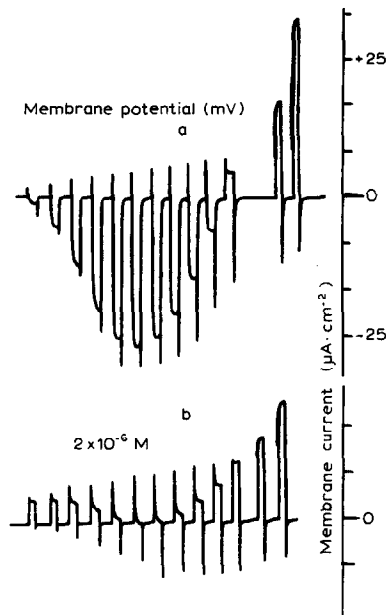


Fig.3. Block of  $I_{Ca}$  of plasmalemma by  $2 \times 10^{-6}$  M polymixin B, introduced to the external solution. The figure shows an original record of transient currents through the plasmalemma  $Ca^{2+}$  channels induced by a pulsed change of the fixed potential,  $V_m$ , from  $-100$  mV to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

**Acknowledgement:** The author is deeply grateful to Professor M.Kh. Chaylakhyan for support of this study.

## 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] Zhereleva, 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] Zhereleva, 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.