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Caffeine releases oscillating opening of calcium-dependent potassium channels in the alga *Eremosphaera viridis*

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Addition of caffeine in presence of 0.1 mM Ca^{2+} releases a prolonged transient polarization of the membrane, indicating a reversible opening of K^+ channels in the plasmalemma of *Eremosphaera viridis*. In the absence of external Ca^{2+} caffeine induces repetitive transient potentials which are inhibited by verapamil. Addition of low Ca^{2+} concentrations prevent these caffeine-induced transient potentials too. These results indicate that caffeine mediates an increase in the concentration of free intracellular Ca^{2+} and an induction of Ca^{2+}-dependent membrane currents, i.e. activation of K^+ channels. The release of Ca^{2+} from intracellular stores may be controlled by cytoplasmic Ca^{2+} levels. A model for the oscillating effect of caffeine is developed.

Caffeine; K^+ channel, Ca^{2+}-dependent; Oscillating transient potential; (*Eremosphaera viridis*)

1. INTRODUCTION

The unicellular green alga *Eremosphaera viridis* shows transient changes of membrane potential and conductance (TP) which are inducible by light-off signals [1] and different chemical effectors [2,3]. Generally the peak of TP corresponds to the potassium diffusion potential (E_K) and reaches its maximum either by hyper- or depolarization, dependent on the relation of the membrane resting potential to E_K [4]. From current- and voltage-clamp experiments [5,6] it is evident that these TPs are caused by a transient, chemical-activated and voltage-independent opening of K^+ channels. Further experiments suggest an activating effect of Ca^{2+} on the opening of these K^+ channels [7,8]. In this paper we describe an effect of caffeine (20 mM) on the Ca^{2+}-dependent activation of K^+ channels in the plasmalemma of *Eremosphaera*. Caffeine is known to evoke Ca^{2+} release from intracellular stores of animal cells by increasing their permeability for Ca^{2+}.

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Abbreviations: E_K, Nernst potential of potassium; ER, endoplasmic reticulum; TEA, tetraethylammonium; TP, transient potential

2. MATERIALS AND METHODS

The coccal green alga *Eremosphaera viridis* was cultivated in nutrient solution II as described by Köhler et al. [1]. For the electrophysiological experiments a cell with an average diameter of about 150 μm was selected for impalement. The measurement of both the transmembrane potential and the membrane resistance was carried out by the computer-supported single microelectrode technique as described elsewhere [1,4]. To determine the membrane resistance the computer generated alternating pulses of 0.1 nA amplitude and 300 ms duration.

The experiments were carried out at pH 5.6 in a medium containing (in mM): 0.1 NaCl, 0.1 $MgCl_2$, 0.1 KNO_3, 0.1 $CaCl_2$ and 2 Mes/NaOH. The Ca^{2+}-free solution was buffered with 0.1 mM EGTA.

The measuring cuvette was perfused at a rate of 2 ml/min by the test solution. Experiments were performed with photosynthetically saturating white light of 150 W/m^2 at a temperature of 20 ± 1.5°C.

3. RESULTS AND DISCUSSION

Ca^{2+}-dependent K^+ channels are widespread in membranes of animal cells [8]. In plant membranes information on the activating effect of Ca^{2+}

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on K^+ channels is steadily accumulating [9]. As previously described the K^+ channels in *Eremosphaera* can be activated by a putative increase of cytoplasmic Ca^{2+} levels [3,10]. In this context a stimulating effect of caffeine (15–25 mM) on the K^+ channels in *Eremosphaera* was investigated. The addition of caffeine to the medium induces TPs in this alga (figs 1,2). However, due to this action it must be distinguished whether Ca^{2+} is present in the external medium.

3.1. *Effect of caffeine in the presence of external Ca^{2+}*

After addition of caffeine (20 mM), following a short depolarization, a single TP with an extremely prolonged plateau phase is released within 1 min accompanied by a strong increase of membrane conductance (fig.1). In comparison with light-off-induced TPs the duration of the plateau phase is extended from 8.8 ± 2.6 s (n = 76) to 87.6 ± 28.8 s (n = 22). This TP can be explained by an activation of K^+ channels in the plasmalemma, because (i) the peak follows exactly the E_K of −175 mV calculated for a given external K^+ concentration of 0.1 mM compared to the control TP and (ii) TEA (10^{-2} mM) suppresses this TP by a direct block of K^+ channels (cf. inhibition of light-off-triggered TPs by TEA [5]).

Caffeine, a methyl derivative of xanthine, has been shown to release Ca^{2+} from isolated sarcoplasmic reticulum of vertebrate skeletal muscle [11]. In intact skeletal muscle fibres caffeine produces both an increase of $[Ca^{2+}]_i$ and a contracture [12,13]. With respect to plants comparable effects of caffeine on intracellular Ca^{2+} stores (ER, vacuole) have been only scarcely discussed [14].

We assume that caffeine evokes Ca^{2+} release from internal reservoirs and in this way mediates an increase in the concentration of free intracellular Ca^{2+} and induction of Ca^{2+}-dependent membrane currents, i.e. long-term activation of K^+ currents. A comparable prolonged TP could be demonstrated by Thaler et al. [3] in *Eremosphaera* under the influence of the calcium specific ionophore A23187. These results indicate that the K^+ channels can be activated by a sustained increased $[Ca^{2+}]_i$ over a long period of time. An interpretation of the caffeine-induced effect may be that caffeine remains bound to a site on the store membrane as long as it is present in the medium,

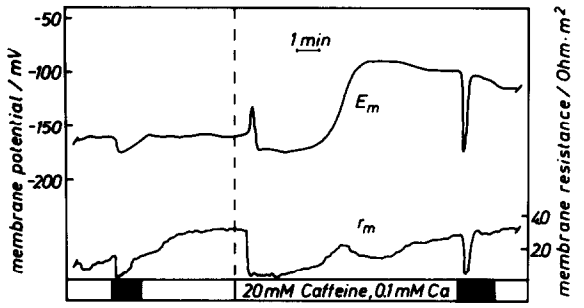

Fig.1. Release of a TP by addition of caffeine (20 mM) to the standard test medium. Before adding caffeine a light-off-triggered TP was released for control. All curves are redrawn from original recordings. $[K^+]_o$ 0.1 mM, $[Ca^{2+}]_o$ 0.1 mM. E_m, membrane potential; r_m, membrane resistance.

activating a Ca^{2+} channel that allows permanent Ca^{2+} efflux as long as there is Ca^{2+} available for release [15]. The fast depolarization after adding caffeine is probably more attained by a change of the donnan and diffusion potential than by a Ca^{2+} influx.

3.2. *Effect of caffeine in the absence of external Ca^{2+}*

In a Ca^{2+}-free medium buffered with EGTA (0.1 mM) caffeine (20 mM) causes periodic opening and closing of K^+ channels (fig.2). These repetitive TPs are characterized by peak values in the range of E_K and an increase of membrane conductance, comparable to the dark-induced TP. On removing caffeine from the medium the repetition subsides immediately. EGTA alone is not able to mediate this oscillating effect. A release of caffeine-induced oscillation is also possible in darkness (not shown). Under the given conditions the oscillation continues for more than 1 h. In the experiment shown it is suppressed instantaneously after the addition of verapamil, a well known organic Ca^{2+} channel inhibitor of animal and plant cells [16]. On the assumption that the receptor site of verapamil in the Ca^{2+} channel is on the cytoplasmic side of the plasmalemma [17] it must be concluded that verapamil is able to permeate biological membranes. In the present experiment its inhibitory effect can be explained with the idea of a caffeine-triggered Ca^{2+} channel in the tonoplast or in ER membranes that become inactive in the presence of verapamil.

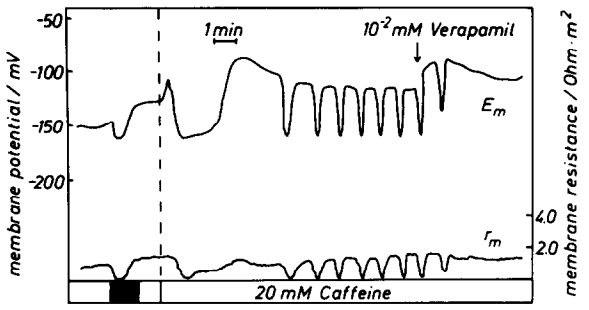

Fig.2. Release of oscillating TPs by addition of caffeine (20 mM) in Ca^{2+}-free medium containing 0.1 mM EGTA. The repetition is suppressed by verapamil (10^{-2} M). In this experiment a control TP in Ca^{2+}-free medium could be released by light-off before addition of caffeine. $[K^+]_o$ 0.1 mM, $[Ca^{2+}]_o$ 0 mM. Further details see fig.1.

Addition of Ca^{2+} at low concentrations to the medium also prevents repetition (not shown). The Ca^{2+} concentration required for complete inhibition ranges from 0.5 to 1 μM in different experiments. At concentrations smaller than 1 μM only the repetition itself and not the amplitude [2] is influenced. The frequency of oscillation is a function of $[Ca^{2+}]_o$. At 0.1 mM EGTA in a Ca^{2+}-free medium the average frequency of repetitive TPs is 0.7–0.8 TP/min. The medium without EGTA has sufficient Ca^{2+} to decrease the frequency to about 0.35 TP/min. An increase in the external Ca^{2+} to 0.5 μM causes a decline of the average frequency to 0–0.2 TP/min.

These results suggest that the oscillation can be influenced by the rate of Ca^{2+} input across the plasmalemma. Thaler et al. [3] recently reported that a short-term influx of Ca^{2+} into the cytoplasm indeed seems to occur in *Eremosphaera* by increasing $[Ca^{2+}]_o$ after Ca^{2+}-depletion. Then Ca^{2+} itself may control the closing of Ca^{2+}-release channels. The involvement of high-affinity Ca^{2+}-binding sites in the control of Ca^{2+}-release is discussed by Argaman and Shoshan-Barmatz [18].

The regulation which leads to repetitive TPs seems to be related with active transport mechanisms (putative a Ca^{2+}-ATPase) located at the plasmalemma and/or tonoplast. A model for a simple feedback mechanism could be developed to explain the repetition: caffeine releases Ca^{2+}, but as $[Ca^{2+}]_i$ is increased it feeds back to inhibit any further release until the influxed Ca^{2+} is transported out of the cytoplasm. Now, caffeine is allowed to initiate another transient. In diverse animal cells oscillations in intracellular Ca^{2+} levels have been found, resulting from the periodic release of Ca^{2+} from internal stores [19]. In this context Ueda et al. [20] monitored periodically Ca^{2+} release which oscillate in phase with the activation of Ca^{2+}-dependent K^+ channels. These results indicate that the mobilization of Ca^{2+} from intracellular stores may be of crucial significance for the releasing mechanism of TPs in *Eremosphaera*. For instance the role of the vacuole as a reservoir for second messenger was recently pointed out by Ranjeva et al. [21].

A considerable advance for the explanation of the periodical stimulation of K^+ channels in the plasmalemma of *Eremosphaera* will be achieved by the use of Ca^{2+}-selective microelectrodes.

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REFERENCES

[1] Köhler, K., Geisweid, H.-J., Simonis, W. and Urbach, W. (1983) Planta 159, 165–171.
[2] Förster, B., Köhler, K., Urbach, W. and Simonis, W. (1989) Planta, submitted.
[3] Thaler, M., Steigner, W., Förster, B., Köhler, K., Simonis, W. and Urbach, W. (1989) J. Exp. Bot. 39, in press.
[4] Köhler, K., Steigner, W., Simonis, W. and Urbach, W. (1985) Planta 166, 490–499.
[5] Köhler, K., Steigner, W., Kolbowski, J., Hansen, U.-P., Simonis, W. and Urbach, W. (1986) Planta 167, 66–75.
[6] Köhler, K., Förster, B. and Kolbowski, J. (1989) Bot. Acta, submitted.
[7] Thaler, M., Steigner, W., Köhler, K., Simonis, W. and Urbach, W. (1987) FEBS Lett. 219, 351–354.
[8] Latorre, R. and Miller, C. (1983) J. Membr. Biol. 71, 11–30.
[9] MacRobby, E.A.C. (1988) Bot. Acta 101, 140–148.
[10] Förster, B., Thaler, M., Urbach, W. and Simonis, W. (1989) in: Proceedings of the 8th International Workshop on Plant Membrane Transport, Elsevier, Amsterdam, in press.
[11] Palade, P., Dettbarn, C., Brunder, D., Stein, P. and Hals, G. (1989) J. Bioenerg. Biomembr. 21, 295–318.
[12] Smith, G.L., Valdeolmillos, D.A., Eisner, A. and Allen, D.G. (1988) J. Gen. Physiol. 92, 351–368.
[13] Su, J.Y. (1988) Pflügers Arch. 411, 371–377.
[14] Tester, M. (1988) J. Membr. Biol. 103, 159–169.
[15] Sorenson, M., Coelho, H.S.L. and Reuben, J.P. (1986) J. Membr. Biol. 90, 219–230.

[16] Graziana, A., Fosset, M., Ranjeva, R., Hetherington, A.M. and Lazdunski, M. (1988) Biochemistry 27, 764–768.
[17] Hosey, M.M. and Lazdunski, M. (1988) J. Membr. Biol. 104, 81–105.
[18] Argaman, A. and Shoshan-Barmatz, V. (1989) FEBS Lett. 243, 88–92.
[19] Berridge, M.J. and Galione, A. (1988) FASEB J. 2, 3074–3082.
[20] Ueda, S., Oiki, S. and Okada, Y. (1986) J. Membr. Biol. 91, 65–72.
[21] Ranjeva, R., Carrasco, A. and Boudet, A.M. (1988) FEBS Lett. 230, 137–141.