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Release of repetitive transient potentials and opening of potassium channels by barium in *Eremosphaera viridis*

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A concentration-dependent effect of Ba^{2+} on voltage-independent opening of K^+ channels in *Eremosphaera* was examined. Low concentrations of external Ba^{2+} (10^{-2} mM) cause the release of repetitive transient potentials. Higher concentrations of Ba^{2+}, however, inhibit these Ba^{2+}-induced repetitive and also light-off triggered transient potentials. Experiments with different Ca^{2+} concentrations in the medium and the treatment with La^{3+} suggest the interaction of Ba^{2+} with Ca^{2+} channels and the participation of internal Ca^{2+} in the activation of K^+ channels.

Ba^{2+} effect; Ca^{2+} channel; K^+ channel; Repetitive transient potential; (*Eremosphaera viridis*)

1. INTRODUCTION

After light-off and addition of different chemical effectors the unicellular green alga *Eremosphaera viridis* shows a rapid transient change of membrane potential and conductance (transient potential, TP) [1]. The peak of a TP corresponds generally to the potassium diffusion potential (E_K) and is independent of cations such as H^+, Na^+ or Ca^{2+} [2]. These TPs are caused by transient, chemical-activated and voltage-independent opening of K^+ channels, which has been demonstrated by current- and voltage-clamp experiments [3,4]. The direction of ion fluxes across the plasma membrane is dependent on the relation of the membrane resting potential to E_K. This paper describes the activating effect of low concentrations of Ba^{2+} (10^{-2} mM) on the opening of K^+ channels in the plasma membrane of *Eremosphaera*. At concentrations of about 1 mM Ba^{2+} is known to be a K^+ channel blocker in animal and plant cells [5–7]. Furthermore, it is suggested that Ba^{2+} is able to replace Ca^{2+} as a physiological effector [8] or to interact with Ca^{2+} channels [9].

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Abbreviations: E_K, Nernst potential of potassium; E_m, membrane potential; TEA, tetraethylammonium; TP, transient potential

2. MATERIALS AND METHODS

For the electrophysiological tests an algal cell (average diameter 145 μm) was selected for impalement. As described in [1,2], continuous measurements of both the transmembrane potential and the membrane resistance were carried out using the single-microelectrode technique. In order to determine the membrane resistance, a.c. pulses of 0.1 nA amplitude and 300–800 ms duration were used.

The external medium contained (in mM): 0.1 $MgCl_2$, 0.1 KNO_3, 0.1 $CaCl_2$. The Ba^{2+} or Ca^{2+} concentrations were changed by adding the chloride salts of these cations. The pH was buffered at 5.6 with 2 mM Mes and the temperature was kept at 20 ± 3°C. The flow rate through the probe chamber was 0.5 l/h. The light intensity was 160 W/m^2.

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3. RESULTS AND DISCUSSION

After addition of Ba^{2+} to the medium, concentration-dependent repetitive TPs in *Eremosphaera* were induced (fig.1). However, due to this effect two ranges of concentration must be distinguished (cf. table 1).

3.1. *Effect of low concentrations of barium*

Ba^{2+} concentrations of 5×10^{-4}–10^{-2} mM (range I) cause complete repetitive TPs, characterized by peak-point values in the range of E_K and a decline of the membrane resistance (fig.1). The duration and release probability of the TPs rise with Ba^{2+} concentration (table 1). At 10^{-2} mM the average frequency of repetitive TPs is 0.6–0.8 TP/min. On removing Ba^{2+} from the medium the repetition subsides immediately. Release of Ba^{2+}-induced TPs is also possible in darkness (not shown). The trigger rate of light-off induced TPs during Ba^{2+} treatment (table 1) seems to be increased (normal rate 50–60%). Within this range of concentration Ba^{2+} has only an activating effect on the excitability of the membrane. The high sensitivity of the cell to activation by Ba^{2+} is independent of E_m within the examined range of -40 to -160 mV as well as of the external K^+ concentration (not shown).

For this effect of Ba^{2+} two basic possibilities can be provided:

(i) Ba^{2+} may be able to permeate via Ca^{2+} channels [10] and directly interact as a Ca^{2+}-imitating ion with K^+ channels [11] or to induce a Ca^{2+}-dependent internal signal chain (calmodulin, phosphorylation). However, generally Ba^{2+} can replace Ca^{2+} as a physiological effector only inefficiently [8,12,13] and therefore an effective Ba^{2+} concentration of 10^{-3} mM seems to be very low, supporting this explanation. In addition, in Ca^{2+}-free medium the release of Ba^{2+}-induced repetitive TPs is suppressed (not shown).

(ii) Consequently, an activation of Ca^{2+} channels by Ba^{2+} seems to be more likely. This effect has also been discussed for the increased excitabili-

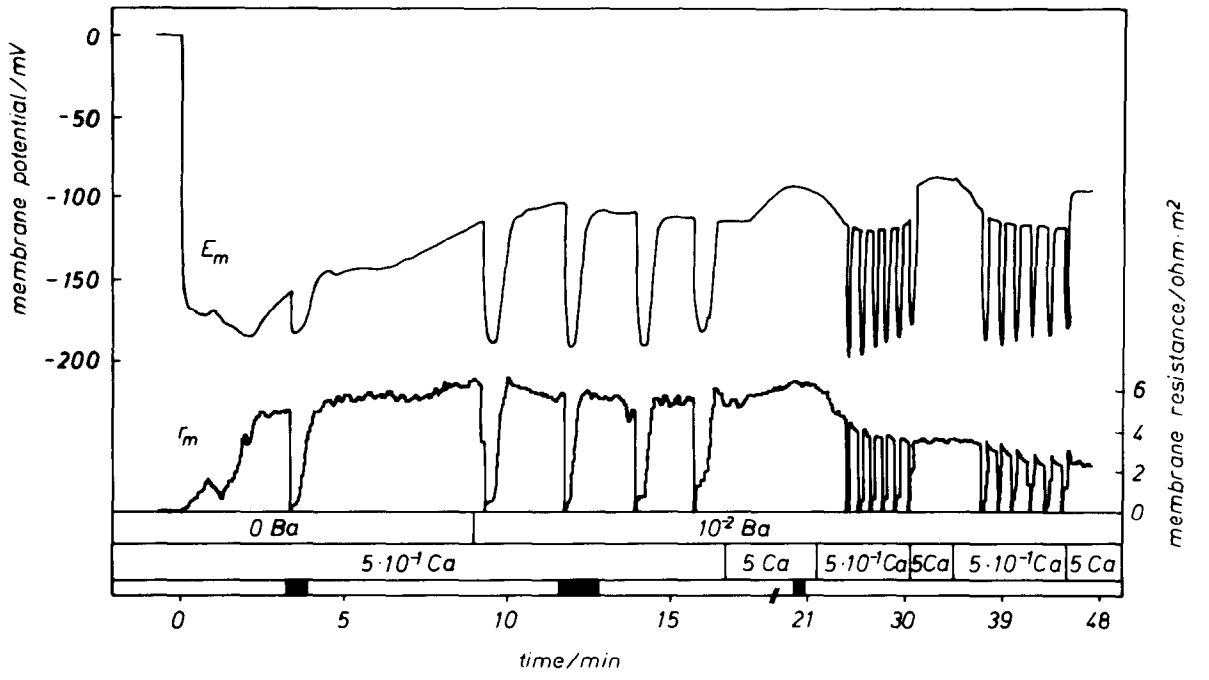

Fig.1. Release of repetitive TPs by addition of 10^{-2} mM Ba^{2+} as a function of external Ca^{2+} concentration. An increase in Ca^{2+} concentration from 0.5 to 5 mM reversibly suppresses the repetitive TPs. Note the change of the time scale after 20 min. All concentrations are given in mM, the light/dark programme is represented as white and black bars and all curves are redrawn from the original recordings. Before adding Ba^{2+}, a light-off triggered TP was released for control. E_m, membrane potential; r_m, specific membrane resistance.

Table 1

Characterization of transient potentials in dependence of different Ba^{2+} concentrations

Range of effect	Ba_o^{2+} (mM)	% release of Ba^{2+} induced TPs	Duration of repetition (min)	% light-off induced TPs after repetition
I	5×10^{-4} (12)	16.6	0.6–3	89
	5×10^{-3} (13)	61.5	3–10	80
	5×10^{-2} (20)	93.7	4–40[a]	80
II	0.1 (12)	95.6	4–25	20
	0.5 (8)[b]	97	2–10	0
	1.0 (10)	100	2–3	0
	5.0 (6)	100	1	0

[a] After max. 40 min Ba^{2+} was removed from the medium
[b] Ba^{2+} concentrations >0.5 mM cause a depolarisation after addition

The external Ca^{2+} concentration was 0.1 mM. Ba_o^{2+}, external Ba^{2+} concentration. Number of experiments in brackets

ty of *Paramecium* on Ba^{2+} treatment [9]. A close connection of the effect of Ba^{2+} with the external Ca^{2+} concentration can be clearly seen in fig.1, where an increase in external Ca^{2+} concentration by a factor of 10 reversibly suppresses the repetitive TPs. The assumption of the participation of Ca^{2+} channels is supported by the inhibition of Ba^{2+}-induced repetition by La^{3+} (not shown), a well-known Ca^{2+} channel blocker [8,14].

3.2. *Effect of high concentrations of barium*

Ba^{2+} concentrations of 0.1–5 mM (range II) have two effects: increasing probability of release of repetitive TPs by increasing concentration of Ba^{2+}, connected with a strong decline in average duration of the repetition (table 1). Fig.2 shows a typical, rapidly subsiding repetition with incomplete TPs, whereas the decline of the peak points runs parallel with the increase in specific membrane resistance. The inhibition of repetitive TPs by Ba^{2+} can be explained by a direct block of K^+ channels (cf. inhibition of light-off induced TPs by TEA [3]). Another possibility may be the disintegration of the internal reaction chain by influx of Ba^{2+}. After the end of the repetition, no further activating effect is observed on increasing the external Ba^{2+} concentration in contrast to concentration range I.

The activating optimum is determined by a specific ion concentration relationship between Ca^{2+} and Ba^{2+} (table 1: 0.01 mM Ba^{2+}, 0.1 mM Ca^{2+}), whereas both ions can serve as activators and channel inhibitory substances [11,15].

These results indicate an important role of Ca^{2+} within the releasing mechanism of TPs in *Eremosphaera*. In animal cells like neurons, muscle cells and erythrocytes, 'calcium gated' K^+ channels have been well investigated [17], whereas chemical activated and voltage independent K^+ channels in plant cell membranes are not described

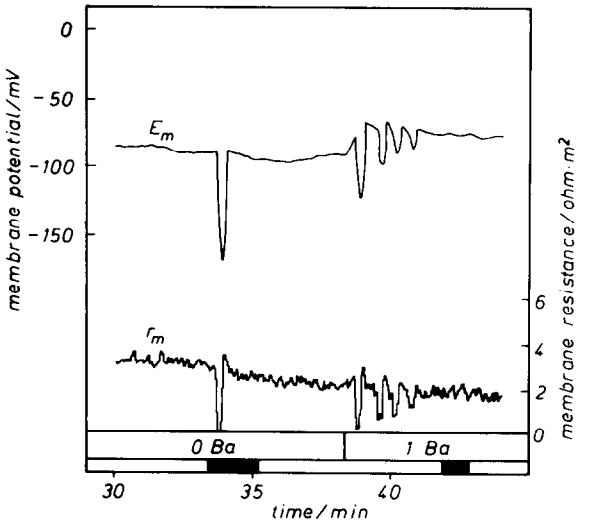

Fig.2. Release of a short repetition of incomplete TPs by addition of 1 mM Ba^{2+}. For further details see fig.1.

so far. Recently, the activation of Cl^- channels in *Characaean* cells during an action potential by Ca^{2+} ions was suggested [12,14,16].

The use of ion selective microelectrodes may provide further insight into the participation of Ca^{2+} within the function of K^+ channels in the plasma membrane of *Eremosphaera*.

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