

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

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

$\text{Ba}^{2+}$  effect;  $\text{Ca}^{2+}$  channel;  $\text{K}^+$  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  $\text{H}^+$ ,  $\text{Na}^+$  or  $\text{Ca}^{2+}$  [2]. These TPs are caused by transient, chemical-activated and voltage-independent opening of  $\text{K}^+$  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  $\text{Ba}^{2+}$  ( $10^{-2}$  mM) on the opening of  $\text{K}^+$  channels in the plasma membrane of

*Eremosphaera*. At concentrations of about 1 mM  $\text{Ba}^{2+}$  is known to be a  $\text{K}^+$  channel blocker in animal and plant cells [5–7]. Furthermore, it is suggested that  $\text{Ba}^{2+}$  is able to replace  $\text{Ca}^{2+}$  as a physiological effector [8] or to interact with  $\text{Ca}^{2+}$  channels [9].

## 2. MATERIALS AND METHODS

For the electrophysiological tests an algal cell (average diameter 145  $\mu\text{m}$ ) 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  $\text{MgCl}_2$ , 0.1  $\text{KNO}_3$ , 0.1  $\text{CaCl}_2$ . The  $\text{Ba}^{2+}$  or  $\text{Ca}^{2+}$  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  $20 \pm 3^\circ\text{C}$ . The flow rate through the probe chamber was 0.5 l/h. The light intensity was 160  $\text{W}/\text{m}^2$ .

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

### 3. RESULTS AND DISCUSSION

After addition of  $\text{Ba}^{2+}$  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

$\text{Ba}^{2+}$  concentrations of  $5 \times 10^{-4}$ – $10^{-2}$  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  $\text{Ba}^{2+}$  concentration (table 1). At  $10^{-2}$  mM the average frequency of repetitive TPs is 0.6–0.8 TP/min. On removing  $\text{Ba}^{2+}$  from the medium the repetition subsides immediately. Release of  $\text{Ba}^{2+}$ -induced TPs is also possible in darkness (not shown). The trigger rate of light-off induced TPs during  $\text{Ba}^{2+}$  treatment (table 1) seems to be increased (normal rate 50–60%). Within this

range of concentration  $\text{Ba}^{2+}$  has only an activating effect on the excitability of the membrane. The high sensitivity of the cell to activation by  $\text{Ba}^{2+}$  is independent of  $E_m$  within the examined range of  $-40$  to  $-160$  mV as well as of the external  $\text{K}^+$  concentration (not shown).

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

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

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

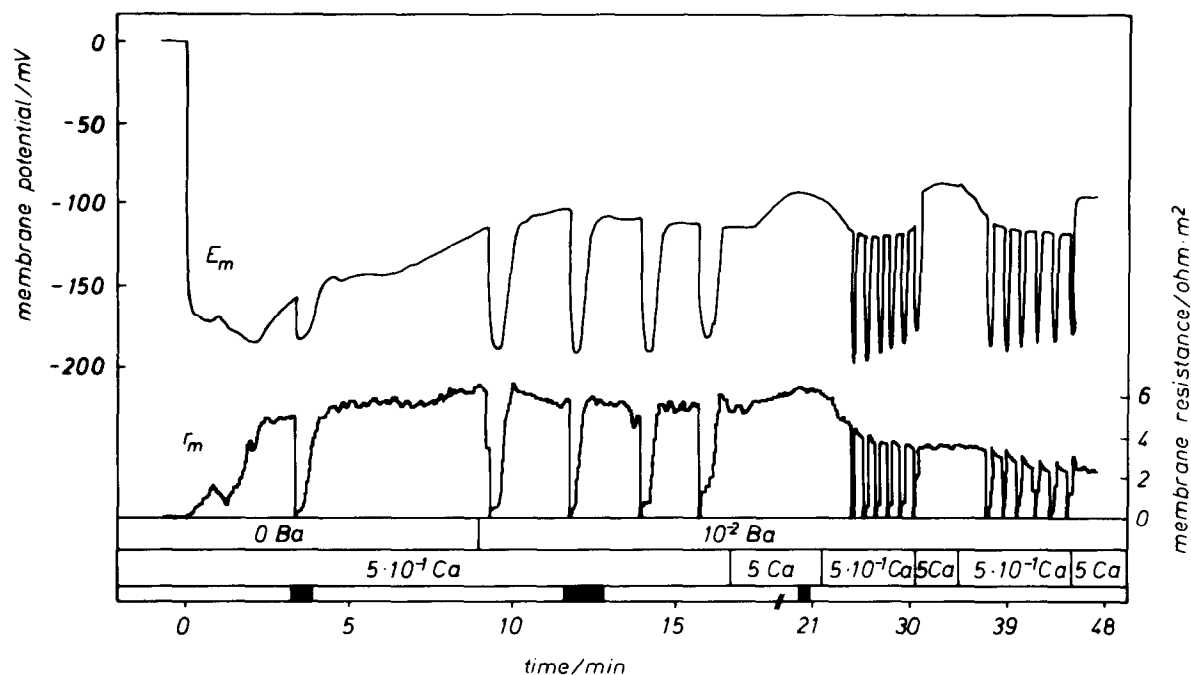


Fig.1. Release of repetitive TPs by addition of  $10^{-2}$  mM  $\text{Ba}^{2+}$  as a function of external  $\text{Ca}^{2+}$  concentration. An increase in  $\text{Ca}^{2+}$  concentration from 0.5 to 5 mM reversibly suppresses the repetitive TPs. Note the change of the time scale after 20 min. All 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  $\text{Ba}^{2+}$ , 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  $\text{Ba}^{2+}$  concentrations

Range of effect	$\text{Ba}_0^{2+}$ (mM)	% release of $\text{Ba}^{2+}$ induced TPs	Duration of repetition (min)	% light-off induced TPs after repetition
I	$5 \times 10^{-4}$ (12)	16.6	0.6–3	89
	$5 \times 10^{-3}$ (13)	61.5	3–10	80
	$5 \times 10^{-2}$ (20)	93.7	4–40 <sup>a</sup>	80
II	0.1 (12)	95.6	4–25	20
	0.5 ( 8) <sup>b</sup>	97	2–10	0
	1.0 (10)	100	2–3	0
	5.0 ( 6)	100	1	0

<sup>a</sup> After max. 40 min  $\text{Ba}^{2+}$  was removed from the medium

<sup>b</sup>  $\text{Ba}^{2+}$  concentrations  $>0.5$  mM cause a depolarisation after addition

The external  $\text{Ca}^{2+}$  concentration was 0.1 mM.  $\text{Ba}_0^{2+}$ , external  $\text{Ba}^{2+}$  concentration. Number of experiments in brackets

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

### 3.2. Effect of high concentrations of barium

$\text{Ba}^{2+}$  concentrations of 0.1–5 mM (range II) have two effects: increasing probability of release of repetitive TPs by increasing concentration of  $\text{Ba}^{2+}$ , 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  $\text{Ba}^{2+}$  can be explained by a direct block of  $\text{K}^+$  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  $\text{Ba}^{2+}$ . After the end of the repetition, no further activating effect is observed on increasing the external  $\text{Ba}^{2+}$  concentration in contrast to concentration range I.

The activating optimum is determined by a specific ion concentration relationship between

$\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$  (table 1: 0.01 mM  $\text{Ba}^{2+}$ , 0.1 mM  $\text{Ca}^{2+}$ ), whereas both ions can serve as activators and channel inhibitory substances [11,15].

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

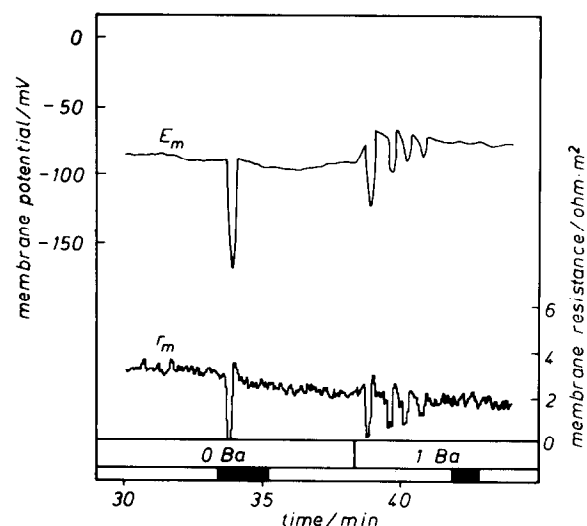


Fig.2. Release of a short repetition of incomplete TPs by addition of 1 mM  $\text{Ba}^{2+}$ . For further details see fig.1.

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

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

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