Effect of K⁺ and Cl⁻ Ion Gradients upon Apex Regeneration in Acetabularia mediterranea*

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Summary. Regeneration of a new apex and electrical activity by anucleate posterior stalk segments (PSS) of Acetabularia mediterranea under imposed transcellular concentration gradients of K⁺ and Cl⁻ has been studied. In a 3 vs. 30 mM K⁺ and in a 26 vs. 545 mM Cl⁻ gradient, respectively, the PSS preferably regenerate at that end exposed to the low K⁺ (replaced by Na⁺) and low Cl⁻ (replaced by SO₄²⁻), respectively. The effect of the K⁺ gradient confirms previous work where voltage-clamped PSS regenerated at the electrically hyperpolarized end (Novák and Bentrup, Planta 108, 227, 1972). The stimulation of regeneration by low Cl⁻, that is, at the depolarized end of the PSS, might be mediated by the electrical impulses arising at this end.

A major conclusion from a previous study of regeneration of the PSS of Acetabularia mediterranea was that the plasmalemma at the presumptive site of regeneration is relatively hyperpolarized by 5 to 10 mV. Similarly, if a PSS is subjected to such a voltage by voltage-clamp, regeneration will start at the hyperpolarized end; furthermore, spontaneous impulses of local depolarization consistently arose at this site (Novák and Bentrup, 1972).

To assess whether ions affect regeneration purely electrically through $E_m$ or the spontaneous impulses, or in a more ion-specific way, PSS have been subjected to transcellular concentration gradients of K⁺ and Cl⁻, respectively. K⁺ gradients have been shown to orient the polarity of Fucus zygotes, presumably through $E_m$ (Bentrup et al., 1967). Similarly, the K⁺ concentration of the medium modifies such orientation by transcellular electrical fields (Bentrup, 1968, 1974). Cl⁻ ions, on the other hand, contribute substantially to the ion conductance of the Acetabularia membrane (Gradmann, 1975).

Preparation of the PSS

Cells of A. mediterranea were cultivated and the PSS obtained as described by Hämmerling and Hämmerling (1959a, b): From 2.5 to 5 cm long cells the basal end with the nucleus containing rhizoid, i.e. about one third of the cell, was cut off and kept 5 days in the dark. Then the rhizoid was also removed and the resulting PSS kept for another day in the dark. Development leading to polar regeneration of a new apex within 40 to 50 h then is elicited by white light (Hämmerling and Hämmerling, 1959b).

The Gradient Experiment

A plexiglass chamber (8 × 8 × 6 cm) was divided by a partition into two compartments. The PSS were fitted into grooves cut into the partition. Ten PSS were held by one chamber. The previous set-up (Novák and Bentrup, 1972) was used, if both, regeneration and $E_m$, were recorded from single PSS. Silicone grease around the PSS prevented bulk flow and provided electrical insulation between the compartments.

Test Solutions

Regular artificial sea water (ASW) was composed as follows (mM): 459 NaCl, 28 MgSO₄·7 H₂O, 25 MgCl₂·6 H₂O, 10 CaCl₂·2 H₂O, 10 KCl, 2 NaHCO₃, and 10 TRIS/HCl to adjust the pH to 7.9. The K⁺ concentration was isosmolarly reduced to 3 or increased to 30 mM by replacing KCl with NaCl. The Cl⁻ concentration was reduced to 26 mM by exchange of NaCl, MgCl₂ and KCl with isosmolar quantities of the respective sulfates.

Development of the PSS in the culture medium (Erd-Schreiber), i.e. without being subjected to any known gradient, consistently showed the existence of a residual cell polarity, that is, the new apex tended to arise preferably at the former apical end of the PSS. On the average, this was observed on 72% of 201 regenerating control PSS. Therefore, the gradient

* Abbreviations: ASW = artificial sea water; $E_m$ = membrane potential; PSS = posterior stalk segment (Hinterstücks).
Table 1. Apex regeneration of anucleate posterior stalk segments (PSS) of *Acetabularia mediterranea* in transcellular concentration gradients of K⁺ and Cl⁻

<table>
<thead>
<tr>
<th>Ion</th>
<th>Compartiment A (mM)</th>
<th>Number of segments</th>
<th>% apex regenerationb in A</th>
<th>Membrane potentialc (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>3 30</td>
<td>46</td>
<td>91 (p&lt;0.1)</td>
<td>-175</td>
</tr>
<tr>
<td></td>
<td>30 3</td>
<td>41</td>
<td>56 (p&lt;0.01)</td>
<td>-155</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10 10</td>
<td>96</td>
<td>80</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>26 545</td>
<td>53</td>
<td>76 (p&lt;0.2)</td>
<td>-131</td>
</tr>
<tr>
<td></td>
<td>454 26</td>
<td>50</td>
<td>42 (p&lt;0.02)</td>
<td>-170</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>545 545</td>
<td>105</td>
<td>65</td>
</tr>
</tbody>
</table>

* Compartment A of the gradient chamber holds the former apical end of the PSS.

b p-values obtained by the χ²-test indicate the reproducibility with respect to the control.
c Average values recorded from at least 9 cells; S.E.M. is about ±2 mV.

experiments were designed with respect to the former cell polarity of the PSS.

Table 1 shows that, in a K⁺ gradient, percent apex regeneration at the former apical end increases from 80 to 91% in 3 mM K⁺, but drops to 56% in 30 mM K⁺. Equivalent is the statement that, for instance, in 3 mM K⁺ percent apex regeneration at the former basal end rises from 20 to 44%. In the Cl⁻ gradient low Cl⁻ enhances the tendency of the PSS to regenerate at their former apical end from 65 to 76% of the population. More clearly, low Cl⁻ promotes regeneration at the former basal end: the figures in Table 1 imply 58% compared to 35% of the control. It should be mentioned that also the percentage of PSS which do not regenerate at all depends upon the ionic situation. While on the average 18% of the intact control PSS in the culture medium did not regenerate, this fraction increased to 37% in the K⁺ gradient, and to 22% in the Cl⁻ gradient.

Finally, Table 1 contains values of $E_m$ for the particular ionic situation. Obviously, low K⁺ hyperpolarizes, and low Cl⁻ depolarizes the *Acetabularia* membrane (cf. Gradmann, 1970). These local changes of $E_m$ have been verified by extracellular recordings from individual PSS during regeneration. Fig. 1 shows the electrical response of a PSS in the K⁺ gradient. The trace consists of the expected steady-state potential of up to +20 mV and a train of repetitive impulses at the depolarized end. Such impulses have been reported previously to arise at the regenerating hyperpolarized end of the PSS (Novák and Bentrup, 1972). Also the Cl⁻ gradient evokes the steady-state $A E_m$ and impulses arising consistently at the site of regeneration. Fig. 1 shows 3 out of a train of 20 in that particular PSS.

Presently, we conclude that the effect of the K⁺ gradient can sufficiently be understood in terms of the steady state $A E_m$, that is, regeneration is promoted at the hyperpolarized end of the PSS, as found previously (Novák and Bentrup, 1972). By contrast, a low Cl⁻ concentration might trigger regeneration by way of the spontaneous electrical impulses. We cannot exclude, of course, ion-specific effects of K⁺ and Cl⁻ independent of the electrical events. Consequently, we currently employ the joint application of voltage-clamp, ion gradient, and isotope flux techniques to assess the role of individual ion species during initiation of regeneration in *Acetabularia*.

Fig. 1. Extracellular recording of the electrical potential difference, $A E_m$, across anucleate posterior stalk segments (PSS) of *Acetabularia mediterranea* regenerating in a K⁺ (upper trace) and Cl⁻ gradient (lower trace) respectively. Positive values indicate that the plasmalemma at the site of regeneration (on the inserted PSS symbols to the left) is relatively hyperpolarized and/or at the opposite end depolarized. For techniques see Novák and Bentrup (1972)