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Rapid Report

Charybdotoxin blocks cation-channels in the vacuolar membrane of suspension cells of *Chenopodium rubrum* L.

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Using the patch-clamp technique, we studied the action of charybdotoxin which blocks Ca^{2+}-activated large-conductance K^+ channels in animal tissue on the slow-activating (SV), Ca^{2+}-activated cation channel in the vacuolar membrane of suspension-cells of *Chenopodium rubrum* L. The toxin reversibly reduced the vacuolar current with $EC_{50} \approx 20$ nM suggesting structural similarities between ion channels in animal and plant membranes.

Passive transport of monovalent cations across the plant vacuolar membrane is largely mediated by a single ion channel species. These slow vacuolar (SV) channels are voltage- and Ca^{2+}-dependent, and have a unit conductance of about 80 pS [1]. They may be blocked by (+)-tubocurarine [2] and activated by calmodulin [3].

Vacuole preparation and patch-clamp measurements were performed as described previously [2]. The test solution contained 100 mM KCl, 2 mM $MgCl_2$, 300 mM mannitol, 1 mM dithiothreitol, 5 mM Tris-Mes (pH 7.2) and 0.1 mM $CaCl_2$, and was used both in the bath and in the pipette. Charybdotoxin was obtained from Latoxan, all chemicals were on analytical grade.

Application of an 100 mV pulse (pipette negative) to a large outside-out-patch of a *Chenopodium* vacuole evoked a slowly saturating inward current. In the presence of 10 nM charybdotoxin on the cytoplasmic side, this current was markedly reduced (Fig. 1). The inward current was lowered in a concentration-dependent manner; half-maximum inhibition was found at about 20 nM (Fig. 2). Single-channel recordings showed that in the presence of 100 nM charybdotoxin the channels were almost completely blocked (Fig. 3).

$K^+(Ca^{2+})$ channels can be classified by their response to the toxins apamin and charybdotoxin [4]. Charybdotoxin is an inhibitor of large-conductance potassium-channels in sceletal muscle [5] and other tissues, whereas apamin blocks $K^+(Ca^{2+})$channels of intermediate conductance [6]). In our experiments, SV channels were only affected by charybdotoxin, whereas 1 μM apamin had no effect (data not shown). These

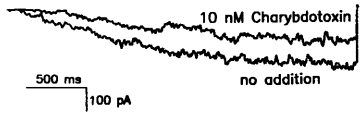

Fig. 1. Effect of 10 nM charybdotoxin on an outside-out-patch of a *Chenopodium* vacuole clamped to −100 mV. Due to the large number of channels in the patch, single-channel openings cannot be resolved.

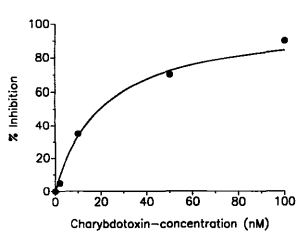

Fig. 2. Relative inhibition of the inward current as a function of the charybdotoxin concentration. Outside-out patches ($n = 4$) were clamped to −100 mV, the average control current was about 300 pA. The concentration for half-maximum inhibition is about 20 nM. The curve was fitted by eye.

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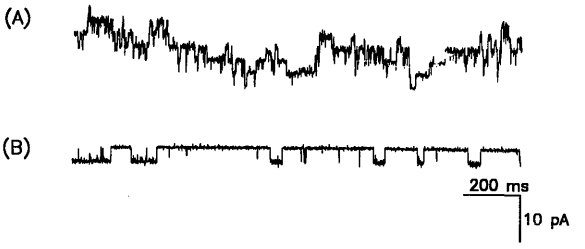

Fig. 3. Single-channel recordings (A) before and (B) after addition of 100 nM charybdotoxin to the cytoplasmic side. The drug drastically lowers the open-probability. Holding potential −60 mV.

findings suggest a close structural relationship between the SV-type cation-channels in the plant vacuolar membrane and large-conductance potassium channels in animal cells.

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