(+)-Tubocurarine is a potent inhibitor of cation channels in the vacuolar membrane of *Chenopodium rubrum* L.

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The effect of the acetylcholine antagonist and channel blocker (+)-tubocurarine on the calcium-dependent slow vacuolar (SV) cation channels in the tonoplast of suspension-cultured cells of *Chenopodium rubrum* L. was examined using the patch-clamp technique. In whole-vacuolar recordings the drug strongly suppressed the potassium conductance (EC_{50}: 6 μM) and altered the kinetics of channel inactivation. In excised membrane patches (+)-tubocurarine evokes channel-'flickering' without affecting the single-channel conductance (approx. 80 pS).

Patch-clamp technique; Plant vacuole; Potassium channel; Tonoplast; (+)-Tubocurarine; *Chenopodium rubrum* L.

1. INTRODUCTION

Passive ion transport across the plant vacuolar membrane, the tonoplast, largely occurs via 'slow vacuolar' (SV) channels [1-3]. These channels preferentially conduct cations, are strongly calcium-dependent, inward-rectifying, and have slow activation kinetics. Up to now only little is known about their pharmacology [4]. In this paper we report the inhibition of SV-channels in the tonoplast of *Chenopodium rubrum* suspension cells by (+)-tubocurarine, a potent blocking agent of some calcium-activated potassium-channels in animals [5-8].

2. MATERIALS AND METHODS

2.1. *Media*

The standard test solution contained 100 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 300 mM mannitol, and 5 mM Tris/Mes (pH 7.2). It was used both in the bath and in the patch-pipette. Test solutions were filtered (Schleicher & Schuell 0.2 μm) just before use. All chemicals were obtained from Sigma.

2.2 *Vacuole preparation and test chamber*

Vacuoles have been prepared from protoplasts isolated from a photoautotrophic suspension culture of *Chenopodium rubrum* L. as described previously [9]. The glass bottom of the perfusion chamber was cleaned with methanol and air-dried. After filling the chamber with the test solution, about 100 μl of a protoplast suspension (approx. 10^6/ml) were transferred to the chamber. Within minutes the protoplasts sedimented and stuck firmly to the glass surface. Perfusing the chamber at a sufficiently high rate broke the protoplasts and released their vacuoles; these likewise were immobilized on the glass surface of the chamber bottom [10].

2.3 *Patch-clamp measurements*

Patch pipettes were pulled from borosilicate glass (Hilgenberg) on a two-stage puller (L/M-3P-A, List Electronics), and heat-polished (microforge L/M CPZ-101, List Electronics). The pipette resistance was about 5 MΩ. The 'whole-vacuolar-mode' was established by sealing the pipette to a vacuole and breaking the patch with a voltage jump (−600 to +600 mV, 10 ms). Thus the vacuolar sap quickly equilibrated with the pipette-solution [11]. 'Outside-out' patches were obtained by withdrawal of the pipette after establishing the 'whole-vacuolar mode'.

Measurements were made using an EPC7-patch-clamp amplifier (List Electronics). Data were stored on videotape after digitizing with an analog/digital converter (VR 10, Instrutech Corp.). After low-pass filtering (1500 Hz) with an 8-pole Bessel-filter (Model 902, Frequency Devices) analysis was performed on an AT-compatible computer using the pCLAMP 5.5 software (Axon Inc.).

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3. RESULTS

Application of a −100 mV voltage pulse of 1.5 s duration to a *Chenopodium* vacuole evoked a large, slowly saturating inward-directed current. A positive pulse of the same amount had only little effect (Fig. 1A). This rectifier property, reported from the vacuoles of other plants [1-3], is not altered by addition of (+)-tubocurarine on the outer, cytoplasmic side of the vacuole (Fig. 1B). In fact, the inward current clearly is inhibited, with half-maximum inhibition (EC_{50}) occurring at about 6 μM (Fig. 2).

In order to investigate the influence of (+)-tubocurarine on single-channel behaviour, measurements on *outside-out* patches were performed. Fig. 3 shows that at 50 μM (+)-tubocurarine causes 'flickering' and markedly reduces open-time of the channels compared to the control, but did not significantly change the single-channel conductance of about 80 pS. Since channel blocking agents may affect the gating of ion channels in different ways [12], we tested whether (+)-tubocurarine changed the kinetics of the macroscopic current. The holding potential across the tonoplast was first for 5 s clamped to −100 mV (vacuole negative) to

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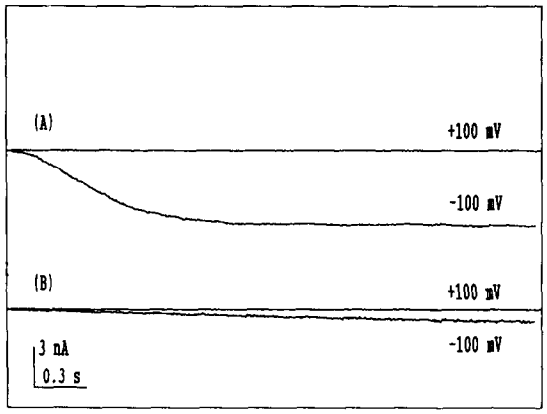

Fig. 1. Whole-vacuole clamp currents recorded from a *Chenopodium* vacuole subjected to the indicated voltages of −100 mV (vacuole negative) and +100 mV, respectively, (A) Control, (B) 50 μM (+)-tubocurarine on the cytoplasmic side.

activate the SV-channels, and then stepped up to +100 mV. Fig. 4 shows tail currents in response to three different (+)-tubocurarine concentrations. Evidently, at 6 or 50 μM, the drug strongly slows down the time course of channel deactivation, whereas at 0.5 μM the current deactivates almost as rapidly as in the control.

4. DISCUSSION

This study demonstrates inhibition of SV-channels in the plant vacuolar membrane by (+)-tubocurarine. In animal cells this plant toxin suppresses calcium-dependent potassium currents. In the guinea pig, 45 μM of the drug inhibits the response of the vas deferens to the hypogastric nerve by 50% [5](the K^+-efflux from hepatocytes is blocked half-maximally by 3.5 μM [6]. Inhibition of the K^+-conductance of inferior mesenteric ganglion cells requires 10–100 μM (+)-

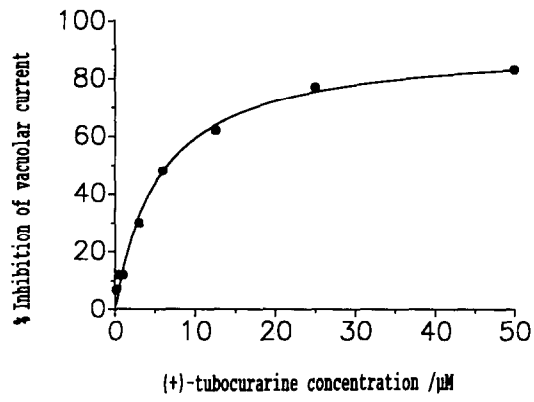

Fig. 2. Relative inhibition of the inward current as a function of the (+)-tubocurarine concentration. Vacuoles ($n=3$) were clamped to −100 mV, the average control current was 5 nA. The curve was fitted according to the Michaelis-Menten formalism, indicating 100% inhibition at 93 μM. Inhibition half-saturates at about 6 μM (+)-tubocurarine.

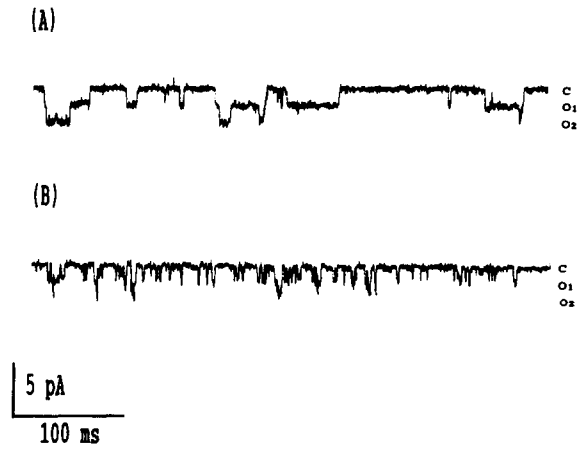

Fig. 3. Single-channel-recordings from an outside-out tonoplast patch derived from a *Chenopodium* vacuole in the absence (A) and presence (B) of 50 μM (+)-tubocurarine. Note the essentially unchanged current amplitudes. C = closed state, O_1 (O_2) = one (two) open states. Holding potential = −20 mV.

tubocurarine [8]. Compared to these values, the effective concentration reported here, i.e. $EC_{50} \approx 6$ μM, suggests a reasonable affinity of the SV-channels in the *Chenopodium* tonoplast to this toxin.

In cultured rat sympathetic neurons, 100 μM (+)-tubocurarine reduced the open-time of a K(Ca)-channel, but did not cause 'flickering' [7]; however, 'flickering' occurred in the presence of 10 μM quinine. We also found quinine-induced channel 'flickering' on the *Chenopodium* SV-channels (data not shown). This comparable inhibition of K(Ca)-channels in animal cells and Ca-dependent potassium-channels in a plant tonoplast by two relatively specific blocking agents seems noteworthy.

We finally address the remarkable effect of (+)-tubocurarine on the vacuolar tail-currents (Fig. 4): with 50 μM (+)-tubocurarine on the cytoplasmatic side, the outwardly flowing tail-current was about three-fold bigger than the maximum inward current of the nor-

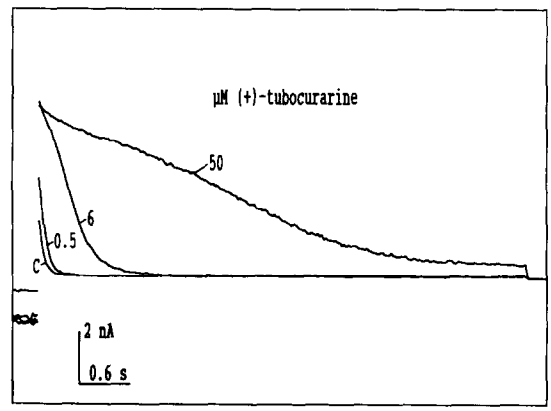

Fig. 4. Influence of 0.5, 6, and 50 μM (+)-tubocurarine, respectively, on tail-current kinetics recorded on a *Chenopodium* vacuole. See text.

mally strongly inward-rectifying channels. Presumably (+)-tubocurarine binds to the activated, open channel and slows down its subsequent deactivation upon depolarization.

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