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A stretch-activated anion channel in tobacco protoplasts

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Stretch-activated ion channels have been described in animal cells, where they might serve as mechanoreceptors, baroreceptors or osmoreceptors, as well as in yeast and bacteria, where osmoregulatory functions have been suggested. Here we report a large conductance, stretch-activated, anion-selective channel in protoplasts of a higher plant, tobacco, and discuss its possible role in osmoregulation.

Stretch-activated channel; Anion conductance; Protoplast membrane; Osmoregulation; Patch clamping

1. INTRODUCTION

Plant cells, like animal cells, transduce physical deformation by external stimuli to changes in plasma membrane ion flux. In plants, the end result is sometimes a change in cell turgor, which produces local movement, or an electrical impulse which may produce action at a distance (review [1]). Animal cells display stretch-activated ion channels in their plasma membranes. Operationally, these are ion channels whose activity in membrane patches increases during application of small pulses of suction to the interior of the patch pipette. First described in cultured skeletal muscle cells [2], stretch-activated channels have been found in mechanoreceptors with external stretch or internal baroreceptor functions (e.g. [3–5]), as well as osmoregulating cells (e.g. [6,7]), some of which are involved in bulk fluid transport. If present in plasma membranes of higher plants, such channels could signal gravity, friction, internal tissue stress or changes in the osmotic pressure of the environment; they could also regulate cell salt content and turgor, as well as growth and movement [1]. Here we present evidence for a large conductance, stretch-activated, anion-selective channel in the plasma membrane of protoplasts from stem-derived tobacco cells grown in suspension cultures, and discuss its possible function in osmoregulation. These data have previously been reported in abstract form [8].

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2. MATERIALS AND METHODS

Protoplasts were prepared from exponentially growing diploid stem cell suspension cultures of *Nicotiana tobaccum* L. cv. Xanthi (original stock a gift of the Monsanto Corp.) maintained as described in [9]. Wall degradation was accomplished by addition of an aliquot of cells to a 'protoplasting' medium consisting of 4% (w/v) CELF cellulose (Worthington Biomedical), 0.01–0.1% (w/v) pectolyase Y-23 (Nissho Iwai), 0.005–0.05% (w/v) bovine serum albumin 25 mM Mes buffered to pH 5.8 with Tris or KOH 500 mM D-mannitol, 5 mM KCl and 10 mM $CaCl_2$. Protoplasting continued for 0.5–2 h, at 22–25°C with slow or intermittent swirling. Without purification, a small aliquot of the protoplasting mixture was released over coverslips which were coated with 0.2% (v/v) polyethylenimine (PEI) and submerged in a large volume of the buffered external salt medium (defined in section 3). Protoplasts sometimes anchored to the coated coverslips via their residual wall remnants, or were restrained by the patch pipettes against a cell wall fragment which adhered to the glass.

Gigaohm resistance pipette-to-membrane seals were formed with 8–20 MΩ resistance pipettes pulled from 100 μl borosilicate glass pipettes (Rochester Scientific). Seals were most successfully obtained by pressing a pipette filled with a solution of 10% lower osmoticum than the bath solution against the protoplast, while applying gentle suction. Single-channel currents were

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recorded and analyzed using a data recording/analysis set-up which we have previously described [10]. Currents were filtered at 0.4 kHz and sampled at 1 kHz. Suction, regulated by a set of valves and reservoirs, was applied through the side port of the pipette holder and was measured using a water manometer connected to the suction line. The clamping potential (V_c) is defined as the negative of the potential applied to the interior of the pipette, with the bath held at ground. Hence, $V_c = 20$ mV depolarizes the patch by 20 mV.

3. RESULTS

The vast majority of cell-attached patches of tobacco protoplasts did not display spontaneous or voltage-induced channel activity. In 50% of tight (< 10 GΩ) patches formed with 200 mM NaCl + 10 mM $CaCl_2$ or 200 mM KCl + 10 mM $CaCl_2$ in the pipette and 5 mM KCl, 10 mM $CaCl_2$ and 0.5 M mannitol in the bath (or holding) solution, application of steps of suction ≤ 10 mmHg to the interior of the pipette evoked long-lasting square jumps of current. The current jumps were seen over a wide range of clamping potentials; current pulses were outward at $V_c = 0$, but reversed direction by $V_c = -50$ mV. Over the range of suction from 10 to 30 mmHg, channel activity at a fixed value of suction rose to a peak within several seconds of application, declined to a lower more constant level for the duration of the suction, and then rapidly disappeared after cessation of the pulse (see fig.1a). The average number of channels open (I/i), in the steady state, increased monotonically with applied suction (fig.1b). Channel activity at a given value of suction did not vary consistently with V_c (fig.1c). From records obtained at several different clamping voltages, it was possible to obtain current vs voltage curves showing consistent zero current potentials of $V_c = -30$ to -40 mV and a tendency to rectify inwardly (i.e. to favor passage of positive current into the cell). Fig.1d shows the single channel conductance to 86 pS for outward current, but 146 pS for inward current. Subconductance states of about 2/3 these values were sometimes seen with prolonged suction.

Stretch-activated channels with very similar kinetics and conductance properties persisted for

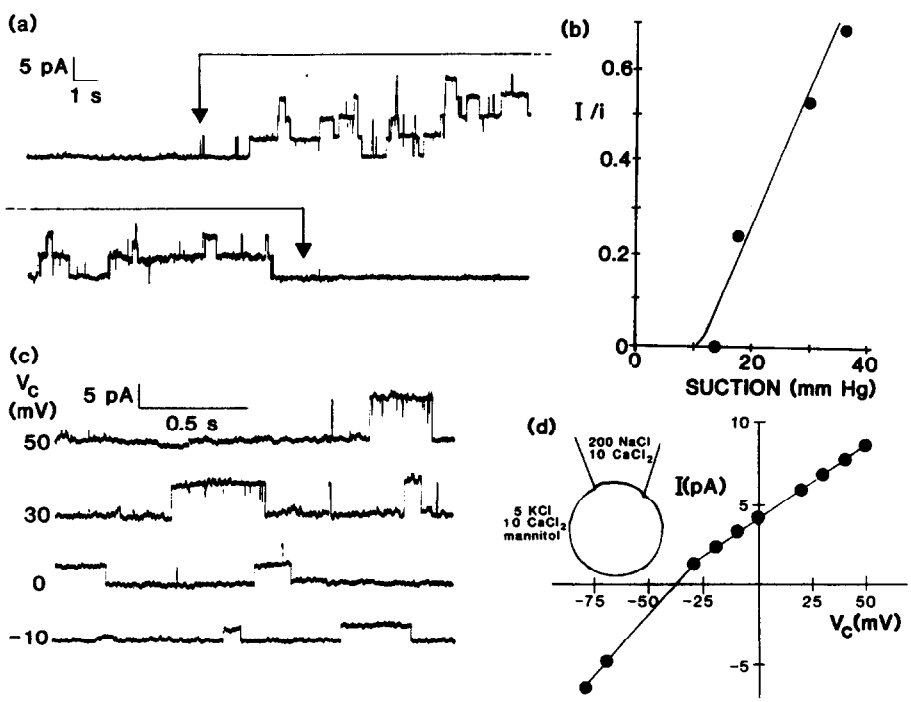

Fig.1. (a) Activation of ion channel in a cell-attached patch of protoplast membrane by application of suction (marked by arrow). (b) Quantitation of average steady-state activity (I/i) of stretch-activated channels in a cell-attached patch as a function of applied suction. (c) Recording of stretch-activated channels at fixed suction but varied clamping potential (V_c). (d) Current vs voltage curve for channel shown in (c). Pipette and bath solutions for a–d are depicted in inset of (d).

minutes after inside-out excision of the stretch-sensitive patch. Fig.2 compares traces of stretch-activated currents in the same patch while cell-attached (a), and several minutes after excision into the bath solution (b). The ion selectivity of the channel is best defined in the excised patch configuration, where it is possible to have known solutions in contact with both the extracellular and intracellular surfaces of the membrane patch. Fig.2c demonstrates that in the presence of an

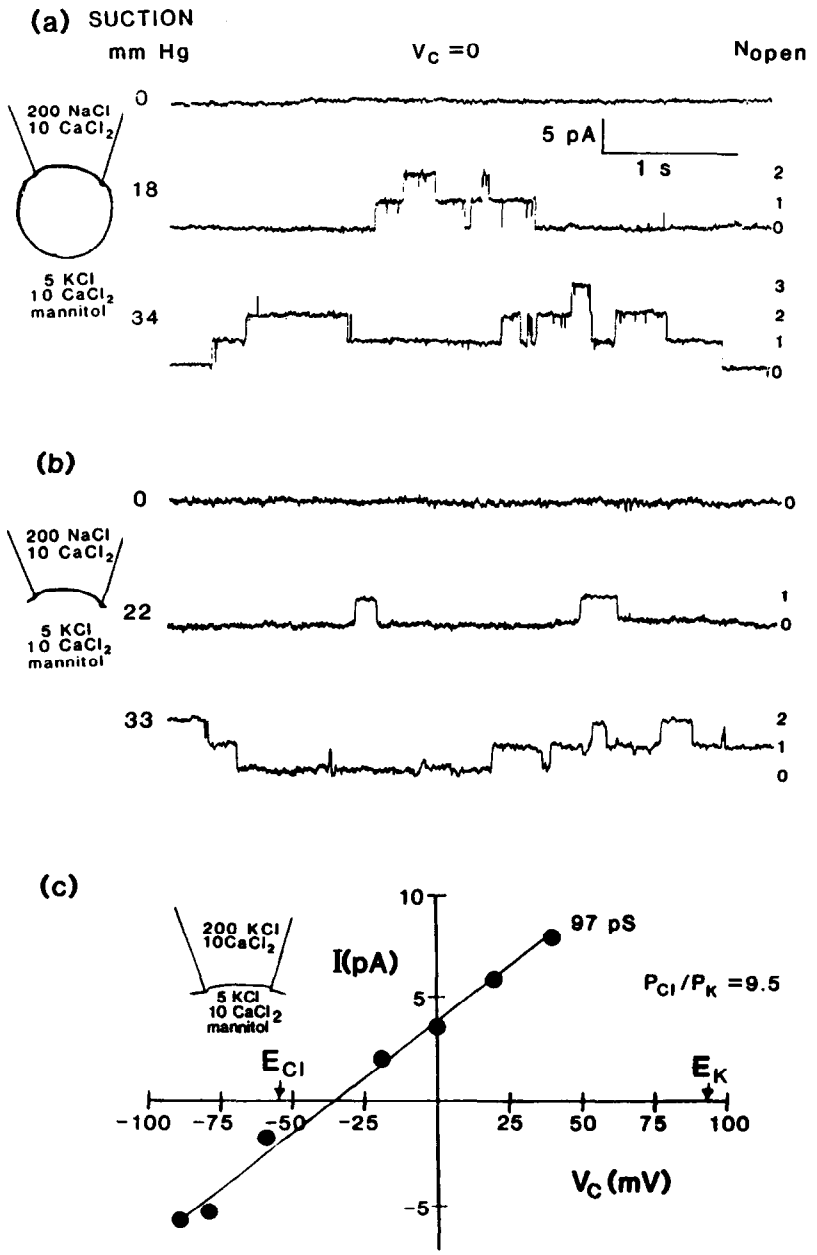

Fig.2. Stretch-activated channel activity recorded during prolonged pulses of suction in a cell-attached patch (a) and then immediately after inside-out excision of the patch into the holding medium (b). Note maintenance of stretch sensitivity in excised patch. (c) Current vs voltage curve of excised stretch-activated channel in the presence of a KCl gradient. E_K and E_{Cl} are marked on the axis. Ambient solutions depicted in inset.

8.8-fold Cl^- gradient and a 40-fold K^+ gradient, both directed inwardly across the membrane patch, the zero current potential (E_{rev}) was -35 mV. This is significantly closer to the Cl^- equilibrium (or Nernst) potential (E_{Cl^-}) than the equilibrium potential for K^+, E_{K+}, indicating a predominant anion selectivity. Ignoring the Ca^{2+} in the bath for the sake of arithmetic simplicity, the Goldman-Hodgkin-Katz diffusion equation can be used to calculate the ratio of Cl^- permeability to K^+ permeability (P_{Cl^-}/P_{K+}) as 9.5.

4. DISCUSSION

Stretch-activated ion channels have now been reported in yeast [11] and bacterial [12] plasma membranes, as well as plasma membranes of a variety of cells in higher animals [2–7]. This report of a large conductance, stretch-activated anion channel in tobacco protoplasts and a preliminary report of stretch-activated cation channels in the protoplasts of guard cells of *Vicia faba* [13] suggest that stretch-activated channels may be present in a variety of plasma membranes of cells of higher plants, along with voltage-gated channels (e.g. [14,15]). The stretch-activated anion channel reported here shows several of the salient features of stretch-activated channels previously reported for other phyla: (i) applied suction of only 10–20 mmHg produces sustained increases in channel activity which subside after cessation of suction; and (ii) enhancement of channel activity by pulses of suction is still possible in inside-out excised patches minutes after patch excision. The latter point suggests that stretch activation is an intrinsic property of the ion channel, or a local, energy-focusing component of the cytoskeleton linked to it [2].

The function of the stretch-activated anion channel during physiological stimulation or stress has not yet been evaluated. The protoplasts have not been subjected to hypo-osmotic shock or tactile deformation beyond the act of gigaseal formation. The similarities between the protoplast's and intact cell's ionic content and response to stress are not immediately clear. Osmoregulation, however, is a phylogenetically old and common property of cells. We suggest that even sparsely distributed stretch-activated anion channels, such as these with large conductances and long mean open times, might contribute significantly to transmembrane anion flux. (A precedent for this may be the pathway underlying the spontaneous pulses of Cl^- efflux and transient depolarizations which occur in the green alga, *Acetabularia* [16], as it swells in hypotonic surroundings.) If a voltage-activated K^+ channel, such as those recorded in a variety of plant cells (e.g. [14,15]), or a stretch-activated cation channel, such as that present in animal cells [2–7], coexists with the stretch-activated anion channel in the tobacco protoplast membrane, then balanced salt exit might be provoked by cell swelling. Preliminary evidence, from an occasional protoplast patch, suggests the possible presence of a stretch-activated cation channel in these cells.

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REFERENCES

[1] Edwards, K.L. and Pickard, B.G. (1987) in: The Cell Surface in Signal Transduction (Wagner, E. et al. eds) pp. 41–66, Springer, Heidelberg.
[2] Guharay, F. and Sachs, F. (1984) J. Physiol. 352, 685–701.
[3] Erxleben, C. and Florey, E. (1988) Pflügers Arch. 411, R155.
[4] Lansman, J.B., Hallam, T.J. and Rink, T.J. (1987) Nature 325, 811–813.
[5] Kirber, M.T., Walsh, J.V. and Singer, J.J. (1988) Biophys. J. 53, 411a.
[6] Falke, L. and Misler S. (1988) Biophys. J. 53, 412a.
[7] Christensen, O. (1987) Nature 330, 66–68.
[8] Falke, L., Edwards, K.L., Pickard, B.G. and Misler, S. (1987) Biophys. J. 51, 251a.
[9] Hauptmann, R.M. and Widholm, J.M. (1982) Plant Physiol. 70, 30–34.
[10] Misler, S., Falke, L., Gillis, K. and McDaniel, M.L. (1986) Proc. Natl. Acad. Sci. USA 83, 7119–7123.
[11] Gustin, M.C., Martinac, B., Saimi, Y., Culbertson, M.R. and Kung, C. (1986) Science 233, 1195–1197.
[12] Martinac, M., Buechyner, M., Delcour, A.H., Adler, J. and Kung, C. (1987) Proc. Natl. Acad. Sci. USA 84, 2297–2301.
[13] Schroeder, J.I. (1988) Biophys. J. 53, 264a.
[14] Schroeder, J.I., Raschke, K. and Neher, E. (1987) Proc. Natl. Acad. Sci. USA 84, 4108–4112.
[15] Satter, R.L. and Moran, N. (1988) Physiol. Plant. 72, 816–820.
[16] Wendler, S., Zimmermann, U. and Rentrup, F.-W. (1983) J. Membrane Biol. 72, 75–84.