

Current-voltage analysis of response to salt stress by salt-tolerant and salt-sensitive charophyte cells

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The electrophysiological responses of salt-tolerant charophyte *Lamprothamnium succinctum* and salt-sensitive charophyte *Chara corallina* to NaCl increase in the medium are compared. The modelling of the current-voltage (I/V) curves allow us to resolve the response of various transporter populations from the total clamp current. The proton ATPase I/V profile is fitted by the HGSS (Hansen, Gradmann, Slayman and Sanders) model of the proton pump (Beilby, 1984). The background current is fitted by an empirical model. The inward K⁺ rectifier in *Lamprothamnium* is modelled by the GHK (Goldman, Hodgkin, Katz) model supplemented by the Boltzmann distribution (Beilby & Walker, 1996).

In both charophytes there is a greater background conductance as Na⁺ concentration in the medium rises. *Lamprothamnium* is able to maintain the negative resting PD (potential difference) by increasing the rate of proton pumping. The HGSS pump model rate constants k_{io}^0 , k_{oi}^0 and κ_{oi} all increase over about 120 min of the salt stress (+ 72 mM NaCl). The inward rectifier activates at more positive PDs.

Chara cells, faced with hypertonic medium of 50 mM NaCl and 0.5 mM Ca⁺⁺ added to the APW (artificial pond water), exhibit more varied responses: (i) pump rate was unchanged, (ii) k_{io}^0 , k_{oi}^0 increased but not κ_{oi} , (iii) k_{io}^0 , k_{oi}^0 and κ_{oi} all increased over 80 min. However, the pumping increase in *Chara* is not enough to keep the membrane from depolarizing. Further, the proton pump becomes very sensitive to both depolarization and hyperpolarization imposed on the membrane. Pump inhibition follows and the recovery is very slow. This instability of the pump impedes recovery of the negative resting PD after voltage clamp to a bipolar staircase command or a spontaneous action potential. It is also impossible to voltage clamp the membrane to sufficiently negative PD levels to investigate the effect of high salt on the inward rectifier.

Charophytes are evolutionarily most closely allied to the green algal line that gave rise to higher plants (Graham & Gray 2001; Karol *et al.*, 2001) and therefore make good models for them. The significance of the differences in one type of transporter rendering the plant cell salt-tolerant can be considered in the light of evolutionary adaptations.

Beilby M.J. (1984) *Journal of Membrane Biology* **81**, 113-125.

Beilby M.J. & Walker N.A. (1996) *Journal of Membrane Biology* **149**, 89-101.

Graham, L.E. & Gray J. (2001) In: Gensel, P.G. & D. Edwards (eds), *Plants Invade the Land. Evolutionary and Environmental Perspectives*. Columbia University Press, New York: 140-159.

Karol, K.G., McCourt R.M., Cimino M.T. & Delwiche C.F. (2001) *Science* **294**, 2351-2353.