Isolated vacuoles of thallus cells of the liverwort *Conocephalum conicum* were studied using the patch-clamp technique. The vacuoles were isolated surgically [Trębacz and Schönknecht, Protoplasma 213 (2000) 39]. In bath and pipette solutions containing: 100 mM KCl, 2 mM CaCl₂, 15 mM Hepes, at pH 7.2, slow vacuolar (SV) currents dominated at positive command potentials. Slowly-activating currents were occasionally recorded at negative command voltages. The probability to activate these currents increased significantly when magnesium, barium or strontium was added to the bath but not to the pipette (in the whole-vacuole and outside-out modes). The currents persisted even after removing Mg²⁺, Ba²⁺ or Sr²⁺ from the bath. The currents were reversibly blocked by zinc (100 µM) and A-9-C (0.5 mM), which are anion channel inhibitors. At a KCl gradient (100 mM in the vacuole lumen and 10 mM in the bath), the currents were significantly reduced. Replacing chloride with gluconate, an impermeable anion, caused the currents to vanish totally. The currents differed from SV, not only in their kinetics, but also in their calcium dependence. They were weakly calcium-dependent and remained active at physiological calcium activities in the cytosol. The probability of recording the currents significantly increased when the isolation procedure was performed in solutions without calcium (EGTA) but with Mg²⁺ at milimolar activity. In the presence of Mg²⁺, Ba²⁺ or Sr²⁺ on the cytoplasmic side of the tonoplast, single channels of 42 pS unitary conductance were recorded at negative potentials. It is concluded that the currents are carried by anion channels. These channels are strongly rectifying. They are suited to conduct anions from the cytosol to the vacuole lumen. The channels can be activated at physiological conditions, submicromolar Ca³⁺ and milimolar Mg²⁺ activities.

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