Interdependence of the Two Borders in a Sodium Transporting Epithelium. Possible Regulation by the Transport Pool

A.W. Cuthbert and W.K. Shum

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD, England

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Summary. Specific binding of $^{14}$C-amiloride to the mucosal surface of frog skin epithelium (Rana temporaria) has been used as a measure of the number of sodium entry sites. All binding measurements were made with the mucosal surface bathed in a solution containing 1.1 mM sodium. When manipulations were used which increased the intracellular concentration of sodium the amount of amiloride bound was reduced. The manipulations included flushing the mucosal surface with solutions containing 111 mM sodium after serosal efflux was inhibited with ouabain or potassium removal. Similar results were obtained when cells were loaded with lithium. These effects on amiloride binding did not appear to depend on changes in membrane potential or upon changes in affinity of amiloride for its binding site. It appears that inhibition of serosal sodium efflux from the epithelium causes a reduction of mucosal sodium influx by making entry sites unavailable. This latter may be a result, directly or indirectly, of the sodium concentration in the sodium transport pool.

The inhibition of sodium transport in frog skin by ouabain, first described by Koefoed-Johnsen (1957), formed part of the evidence upon which the two-series barrier model of epithelial sodium transport (Koefoed-Johnsen & Ussing, 1958) was based. The Ussing model, as it has become known, has dominated thinking on epithelial transport processes for two decades. During this time there have been numerous studies designed to look at the properties of the two-series barriers individually, that is, the entry step at the mucosal surface and the exit step at the serosal side.

Arguments which favor an action of ouabain at the serosal surface of sodium transporting epithelia are extensive. For example, the glycoside is effective only when applied to the serosal surface (Bonting & Canady, 1964; Asano et al., 1970) and Na-K ATPase has been demonstrated histochemically at the basolateral borders (Keller, 1963; Farquhar & Palade, 1966; Mills & Ernst, 1975).
Recently evidence suggesting that inhibition of the serosal sodium pump modifies the properties of the mucosal face has been obtained. It was found in frog skin that preincubation with ouabain reduced sodium uptake at the mucosal surface (Biber, 1971), but only if the preincubation was carried out in sodium-containing solutions (Erlij & Smith, 1973). Conductance measurements in toad skin indicate that the sodium conductance of the apical membrane is decreased by inhibition of the serosal pumping mechanism (Larsen, 1973), as is the uptake of sodium through the apical surface of toad bladder (Finn, 1975). Changes in intracellular sodium concentration or of potential across the apical membrane have been suggested as the cause of altered apical membrane permeability.

We have attempted to measure the properties of the apical membrane more directly under both control conditions and after transport had been inhibited with ouabain. To do this we have used frog skin (Rana temporaria) and have made binding measurements with $^{14}$C-amiloride, an agent known to inhibit sodium entry through the mucosal face by combination with the translocation mechanism (Bentley, 1968; Ehrlich & Crabbé, 1968; Dörge & Nagel, 1970; Nagel & Dörge, 1970; Salako & Smith, 1970a–b). It has been found that procedures designed to increase the intracellular concentration of sodium reduce the number of sites which can be labeled by a given amiloride concentration. Other experiments indicate that it is unlikely that increased intracellular sodium leads to substantial changes in affinity for amiloride. Thus, the findings might indicate that sites for sodium entry become increasingly unavailable as the intracellular sodium concentration is raised.

**Materials and Methods**

All experiments were performed on the abdominal skins taken from frogs, Rana temporaria, stored in tanks at room temperature. Binding of $^{14}$C-amiloride was measured as described elsewhere in short-circuited skins (Cuthbert, 1973; Cuthbert & Shum, 1974a–b; 1967a–b). Binding was measured with the mucosal surface bathed in low sodium Ringer's (1.1 mM), while the serosal solution was bathed in normal Ringer's solution (111 mM Na$^+$). This procedure increases the apparent affinity of amiloride (Cuthbert, 1973; Cuthbert & Shum, 1974b) and is an essential requirement with this ligand for the detection of specific binding. Throughout the specific binding has been taken as the difference between the amount of radiolabel retained in the presence of amiloride ($10^{-8}$ M and 54 C/mole) and that retained in the presence of amiloride ($10^{-6}$ M and 0.54 C/mole). Skins (9.6 cm$^2$) were short-circuited throughout with a Schema Versetae, 360-e voltage clamp. Previously we have stated binding results as binding sites/μm$^2$ of mucosal surface. To derive these values we have used the product of the amount bound and the reciprocal of the fractional occupancy. The fractional occupancy