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ON THE AMOUNT OF (Na^+ + K^+)-ATPase AVAILABLE FOR TRANSEPITHELIAL SODIUM ION TRANSPORT IN THE AMPHIBIAN SKIN

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Summary

Pretreatment of frog skin epithelium homogenates with sodium dodecyl sulphate in the presence of ATP reveals levels of ouabain-sensitive ATPase activity usually higher and occasionally far higher than those required to sustain maximum rates of Na^+ transport. This supports the view that Na^+ transport involves only a fraction of the epithelial cells.

The amphibian skin can perform an uphill transport of Na^+ from the external medium towards the plasma or internal medium, both in vivo and in vitro. Earlier models of the mechanism responsible for this transport involve all the layers of cells in the stratified skin epithelium [1—3] but there is a growing body of evidence pointing towards a single cell layer, probably the upper layer of cells of the stratum granulosum, being responsible for the overall transepithelial transport of Na^+ [4,5].

A main stumbling block in the way of the single-cell-layer theories is the observation that the total amount of (Na^+ + K^+)-ATPase available in the whole thickness of the skin [6] or in the epithelium [3] is only just enough to account for observed rates of transepithelial Na^+ transport. This is particularly striking if one considers that the enzyme assay was performed under near optimum conditions while the Na^+ transport values used for comparison [3,6] correspond to sub-maximal rates of transport. This "ATPase deficit" could be genuine or the result of enzyme exclusion. This could be due to the fact that frog skin homogenates are not really homogeneous [6], thus limiting the accessibility of co-factors and substrate to the enzyme during the reaction.

We report here the finding that pretreatment of frog skin epithelium homogenates with the detergent sodium dodecyl sulphate reveals levels of ouabain-sensitive ATPase activity usually higher and occasionally far higher than those required to sustain the rate of transepithelial Na^+ transport measured

before the homogenizing procedure in the same area of isolated skin epithelium.

Sodium dodecyl sulphate was recently used by Jorgensen [7,8] to obtain a higher yield of purified ($Na^+ + K^+$)-ATPase from the outer medulla of the mammalian kidney. According to this author, sodium dodecyl sulphate selectively removes extraneous lipid and protein components from the membrane thus rendering the enzyme associated with the microsomal vesicles more accessible to the co-factors of the enzymic reaction, increasing thereby the specific activity without further purification. The presence of ATP provides some protection of the enzyme against inactivation by sodium dodecyl sulphate [7,8].

The abdominal skin from *Rana temporaria* was removed and about 3.5 cm^2 of epithelium were dissected out from the underlying corion following the technique of Aceves and Erlij [9] modified in the following way. The skin was mounted as a flat sheet on a 20 cm^2 perspex funnel, with the corion side towards the stem. The epithelial side was immersed in a beaker with Ringer at 22—25°C, stirred magnetically. 5 ml of Ringer containing 80 units of collagenase per ml were put in contact with the corion side for 1.5—2 h. After this, a gentle pressure was applied on the corion through the tube until a blister was formed. This took about 1—5 min. The dissection was started at the blister and completed in about 2 min.

The isolated epithelium was mounted between two symmetrical lucite chambers containing Ringer with the following composition: NaCl 112 mM, KCl 2.4 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, glucose 5 mM, Tris base was added to give a final pH of 8. Short circuit current (I_{sc}) (which in *Rana temporaria* equals the net Na^+ transport in the absence of electrochemical gradients) and membrane conductance were recorded continuously at 19—20°C. Preliminary experiments showed that the dissection procedure had virtually no effect on the I_{sc} and membrane conductance of the isolated epithelium.

After 1 h the two half chambers were separated, and the exposed part of the epithelium was cut out, gently blotted on filter paper, weighed and homogenized by hand with a glass grinder in 2 ml of ice-cold solution containing: Na^+-ATP 3 mM, Tris/EGTA 2 mM and imidazole 50 mM (pH 7.5). Since the stratum corneum of the skin has a high Ca^{2+} content, the presence of excess EGTA proved to be necessary to avoid inhibitory levels of Ca^{2+} in the final reaction medium. The average weight of the isolated epithelia was about 20 mg. This made the homogenizing procedure by other methods extremely inefficient and glass grinding was considered the best choice.

The homogenate was divided into two 1-ml portions and to one of them sodium dodecyl sulphate was added to give the final concentration of 0.1 mg per ml. The suspensions were subsequently incubated for 15 min at 20°C with continuous magnetic stirring. 25—50-μl aliquots of both control and sodium dodecyl sulphate-treated enzyme suspensions were added to 1 ml of medium containing; Tris/EGTA 0.5 mM, $MgCl_2$ 2.5 mM, [γ^{32}P]ATP 2 mM, Tris·HCl (pH 7.5) 5 mM, NaCl 120 mM and KCl 12 mM with or without ouabain $1 \cdot 10^{-2}$ M, and incubated at 25°C for the indicated times. The ATPase activity was estimated by measuring the inorganic phosphate released and trapped as [^{32}P]phosphomolybdate in an isobutanol phase, after inactivation of the enzyme with trichloroacetic acid.

The use of this rather crude preparation was justified by the need to assess all the measurable ouabain-sensitive ATPase activity that could be recovered from the area of membrane where the I_{sc} had been measured before. Any attempt at purification whould have decreased the yield of enzyme to an unmeasurable extent rendering the comparison between active sodium transport and ouabain-sensitive ATPase meaningless.

The skin homogenate even in the absence of corionic tissue, was extremely lumpy and non-homogeneous, the lumps representing clusters of intact cells as seen under the microscope. Similar difficulties were faced by Bonting and Caravaggio [6], with whole skin homogenates, which probably accounts for the very low values of ouabain-sensitive ATPase reported by these authors. This lumpiness introduced a large scatter in the results and by effectively excluding part of the epithelium from the enzyme assay led to an underestimation of the true ouabain-sensitive ATPase activity. In three out of twelve experiments we failed to detect any significant amount of ouabain-sensitive ATPase activity altogether. Since sodium dodecyl sulphate also inactivates the $(Na^+ + K^+)$-ATPase even under near optimum conditions, the treatment of the preparation with sodium dodecyl sulphate could only have reduced the total amount of $(Na^+ + K^+)$-ATPase while at the same time increasing its specific activity. This might have contributed to further underestimation of the total enzyme activity.

Fig. 1 shows a representative result obtained with a skin having a I_{sc} value of 170 μA/3.14 cm^2 and a conductance of 2.5 mΩ^{-1}/3.14 cm^2 just before the enzyme assay.

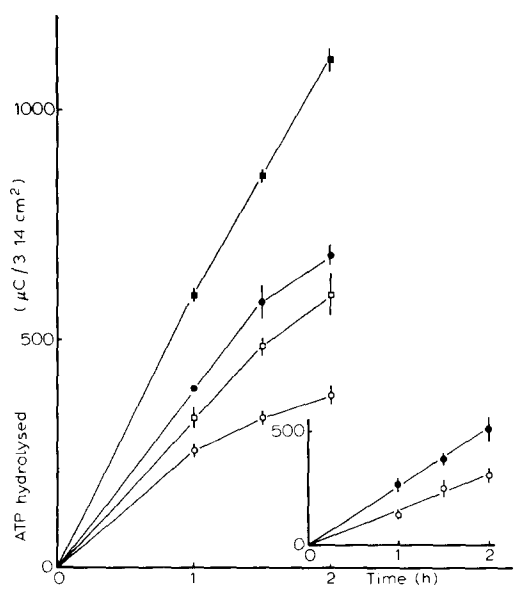

Fig. 1. Time course of ATP hydrolysis by homogenates of frog skin epithelium in the presence and absence of ouabain. Insert: ouabain sensitive ATP hydrolysis. The ordinates give the number of μC per 3.14 cm^2 equivalent to the amount of ATP hydrolyzed assuming a stoichiometry of three Na^+ transported per molecule of ATP hydrolysed. The short circuit current of this skin before the enzyme assay was 170 μA per 3.14 cm^2. □, control; ○, control with 10 mM ouabain; ■, sodium dodecyl sulphate-treated enzyme; ●, sodium dodecyl sulphate-treated enzyme with 10 mM ouabain; Insert: ○, control; ●, sodium dodecyl sulphate-treated enzyme. Each point represents the mean and standard error of triplicate samples.

Fig. 2 summarises the results obtained with all twelve skins tested.

It can be seen that while the ouabain-sensitive ATPase activity of untreated skins is generally comparable to the I_{sc} measured in the same skin, that of the sodium dodecyl sulphate-treated epithelium is usually much higher. In nine out of twelve skins sodium dodecyl sulphate increased the ouabain-sensitive ATPase activity with variable effects on the ouabain-resistant components. Considering that the measured ATPase activity could only have been underestimated for reasons discussed before, the fact that some individual values exceeded the maximum rates of Na^+ transport observed in this preparation suggests that there is more enzyme available than that required for the transepithelial transport of Na^+. This is consistent with the view that only a fraction of the epithelial cells might be responsible for the active transport of Na^+ across the whole skin.

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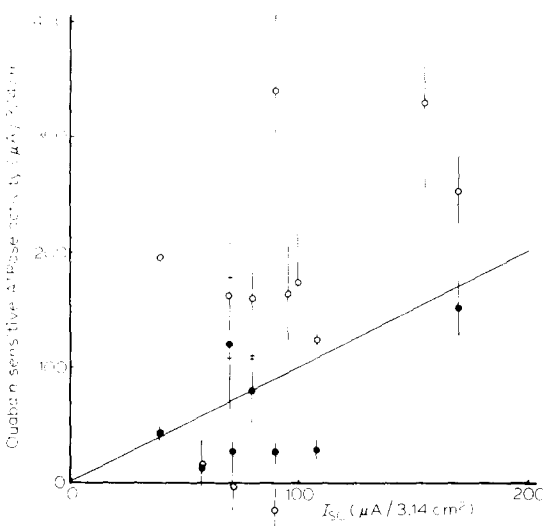

Fig. 2. Comparison between the ouabain-sensitive ATPase activity and the short circuit current in the same area of isolated frog skin epithelium. The continuous line represents the identity line. •, controls; ○, sodium dodecyl sulphate-treated enzyme. Each point represents the ouabain-sensitive ATPase activity (slope of regression lines like those from insert of Fig. 1) ±, the standard error of the slope. Note that the enzyme activity here is expressed in μA per 3.14 cm^2.

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