# ASPECIFIC ALKALINE PHOSPHATASE OF AMPHIBIA INTEGUMENT LEVAMISOLE EFFECT ON SHORT CIRCUIT CURRENT (SCC)

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## INTRODUCTION

For years, the frog skin has been the guiding model for ion transport processes in animal cells energized by a $Na^+$-motive force. In fact, frogs are faced with various osmoregulatory problems, such as compensation of salt and water loss or metabolic acidification. Being exposed both to air and to pond water of low salinity in their natural habitat, the epithelium of the frog skin serves as one of the major organs for body fluid homeostasis.

In many previous works we have observed histochemical variations of alkaline phosphatase (alPase) activity in the integument of some amphibian species. These variations are supposedly associated with the transport of ions through the integument. The enzyme activity modifications appear linked with variations of the active transport of ions, as measured in an Ussing chamber (SCC) (1,2).

The skin of amphibia is characteristically heterocellular, containing two major cell types: principal cells (PC), and mitochondria-rich cells (MRC). The latter cells are interspersed between the principal cells, at the outer (apical) side of the epithelium. The principal, epithelial cells of the amphibian skin are engaged almost exclusively in the active transport of sodium, while the MR cells are the major site for chloride movement and proton secretion (3).

The frog skin is capable of active transepithelial $Na^+$ absorption which is matched by $H^+$ secretion. The regulation of transport involves insertion

of $H^+$ pumps (exocytosis) from a cytosolic pool into the apical membrane (rapid insertion of vesicles containing preformed V-ATPase holoenzymes into the luminal membrane). A dynamic control of acid secretion may be achieved by exocytotic insertion and endocytotic retrieval of V-ATPase pumps, so Aldosterone induces the insertion of new membrane containing functional $H^+$ pumps into the apical membrane of MRCs.
The $Na^+$ absorption from diluted solution via PCs is energized by the activity of the $H^+$ pump. $Na^+$ uptake (through Amiloride-blockable $Na^+$ channels) occurs essentially through PCs and proton pumps are localized in the apical membrane of PCs.
According to Baker and Hillyard (4), the natriferic response in the frog skin is associated with an increase in the density of $Na^+$ channels in the apical membrane.
In polarized epithelial cells newly synthesized membranes or secreted proteins are probably sorted in either the trans-Golgi network or the post-Golgi into different vesicle populations to be delivered to the different membrane domains (5). Alkaline phosphatase and membrane dipeptidase are endogenous GPI anchored proteins, that have been characterized as predominantly brush border apical membrane proteins.

## MATERIALS AND METHODS

Freely swimming Rana esculenta complex specimens from a commercial dealer were used. The anaesthesia was performed in MS 222 Sandoz, acqueous solution (0.1%) used at room temperature. A lateral-abdominal skin portion was dissected, rinsed in amphibian Ringer and then mounted on an "Ussing-type" chamber (5 x 10 mm), with silicone gaskets to minimize edge damage. Both sides were bathed in aerated Ringer's solution (mM composition: NaCl, 90; $NaHCO_3$, 25; KCl, 3; $CaCl_2$, 1; $MgSO_4$, 0.5; $KH_2PO_4$ 0.5; glucose 5.5), pH about 7.6.
Potential difference (PD) across the skin was measured by means of calomel electrodes and salt agar bridges connected to a DC amplifier and a millivolmeter. Current was applied across the skin through an automatic voltage clamp, Ag-AgCl cells and salt agar bridges. Short circuit current

(SCC) was determined manually adjusting PD to zero (6), and then continuously recorded.

The contribution of alPase in skin transport phenomena was controlled by adding -L-Levamisole (Sigma, CAS[16595-80-5]) in different and graded concentrations: 0.0025, 0.025, 2.5 e 25 mM.

RESULTS

Levamisole 0.025 mM, added to Ringer's solution bathing the exterior side of the integument doesn't produce any kind of effect. Levamisole 0.025 mM induces an SCC increase, thus indicative of the activation of ion transport; a higher concentration (2.5 mM) increases further the SCC value. Levamisole 25 mM produces a rapid, but transitory, increase of SCC, immediately followed by a decrement. The higher concentration (25 mM) seems to give an irreversible effect because the functional character is lost also after several and prolonged washings with Ringer's solution (fig. 1).

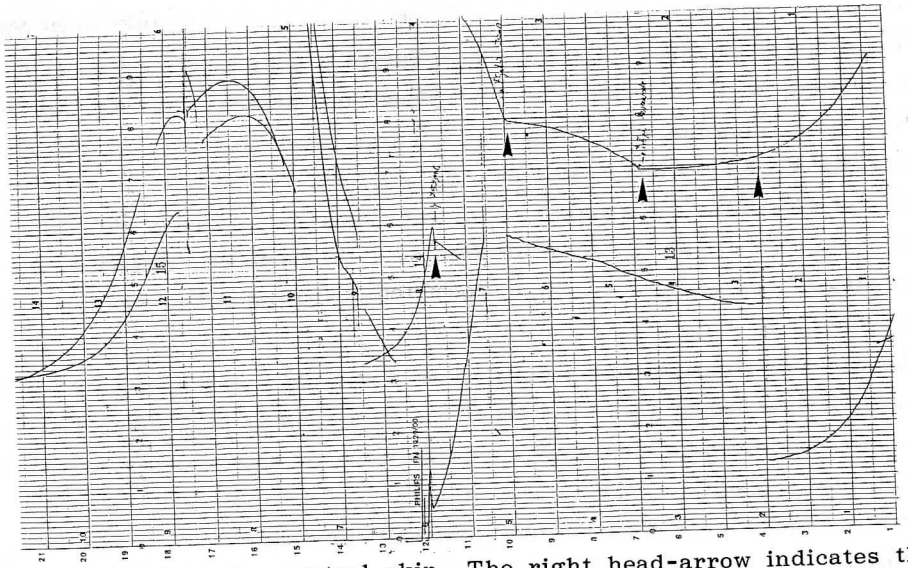

Fig. 1 - SCC in the ventral skin. The right head-arrow indicates the addition of Levamisole to a final concentration of 0.0025 mM in the media. Subsequently the effects of the addition of Levamisole to reach a concentration of 0.25, 2.5 and 25 mM are recorded.

Similar results are achieved working, in the same conditions, with the interior side of the integument.

## DISCUSSION

The inhibition of alPase activity using Levamisole, a "specific" inhibitor, resulted in SCC increments. The final concentrations in the media bathing the surviving epithelia are similar to those effective as alPase enzyme inhibitor both in tissue homogenates and tissue sections.

Meaningful interpretation of the data requires a histochemical knowledge about the cellular location of the specific transport mediators, since net ion flux depends on the integrated activity of several different cell types. The transport properties of polarized epithelial cells depend on the subcellular location of specific ion transport mediators in the cell's apical and basolateral membrane domains.

The outermost living cell layer of epithelium in amphibian, mostly formed by the apical membrane of PCs, is characterised by alPase activity (7); almost exclusively in this membrane the $Na^+$-amiloride sensitive, selective channels are localised. Alkaline Phosphatase is a broad and general term associated with non-specific phosphomonoesterases, having optimal activity at alkaline pH. AlPase is a ubiquitous enzyme, present from bacteria to man; although this enzyme has been extensively studied, the physiological substrates and its biological relevance are poorly understood. The substrates used to reveal by histochemical method or to quantify the activity of the enzyme are highly toxic and optimal pH is far from the physiological range.

The microenvironment of an epithelium is the part of the outside world that is influenced by the activities of the epithelium and, in turn, dictates the circumstances under which the epithelium must operate. In Rana, such as in other amphibia, AlPase and other glycoproteins are loosely linked to the external layer of membrane lipids and represent the outermost "non stirred" exchange surface of the skin, directly exposed to the water. We propose that alPase may be considered as an "auxiliary transport protein" that in some ways facilitates transport of various substrates

across plasma membrane, excluding direct participation of the enzyme to the transport. AlPase may cycle between the membranes of the cytoplasmic organelles and the cell surface, where the enzyme is active to create the particular conditions required for the activity of some of the specific carriers for the various transported molecules. Thus the enzyme may play a structural role in the formation of complex between specific transporters and substrate or serves a regulatory function.

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Using the Ussing chamber technique we have measured the short-circuit current (SCC), and so the ion transport, in the ventral skin of samples of Rana esculenta complex. The animals were not exposed to experimental treatment, and on SCC we have observed the effect of levamisole, administered either on external or internal side.
Levamisole 0.0025 mM was ineffective; higher concentrations (0.025 mM, 2.5 mM), which inhibit alPase activity in tissue extracts and sections, induced an increase in SCC measurements and the effect was proportional to the concentration. Levamisole 25 mM produced a rapid and transitory increase of SCC, followed by a very quick decrement of it.
Because of the action of Levamisole, "specific inhibitor of alPase activity", on ion transport in Rana skin, we propose that the alPase enzyme is probably involved in ion cutaneous transport and thus in the adaptative osmoregulation in the integument of amphibia.

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