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# Staphylococcal alpha toxin induced ionic transport and permeability changes in frog skin

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STAPHYLOCOCCAL alpha toxin (ST), one of the most active staphylococcal products changes and eventually damages membranes of erythrocytes,[1] thrombocytes[2] and mast cells.[3] The frog skin was chosen as a further model to study the membrane effects of the toxin. The technique of Ussing and Zerahn[4] that we used, enables to determine the changes of ionic transport and of permeability of amphibian membranes by measurement of electrophysiological parameters. The potential difference (PD) and short-circuit current (SCC) were checked. 119 mM Ringer's solution bathed the inside of the skin (corium), whereas 20 mM NaCl bathed the outside (epidermis). The replacement of $Na^+$ by $K^+$ on the outside of the untreated skin led to the loss of PD and SCC,[5] as only $Na^+$ is actively transported from the outside across the skin. Unpurified ST was added to the inside.

After ST administration PD and SCC were depressed similarly for about 20 min. Only SCC raised again after a varying period of time (Fig. 1). Full replacement of $Na^+$ by $K^+$ in the outside medium for 10 min did not cause any substantial change of PD and SCC nor did the replacement of $Na^+$ by $Ca^{++}$, $Mg^{++}$ and choline chlorides. Thus the ST effect was characterized by unchanged PD and high SCC values regardless of the type of cation present in the outside medium. This pointed at an impairment of either ionic transport or membrane permeselectivity.

Therefore net $Na^+$ flux on ST treated skin using isotopes $Na^{22}$ added to the outside and $Na^{24}$ added to the inside was measured. The results are presented in Table 1. It demonstrates that net flux of $Na^+$ was reversed in the direction, from inside to outside. Thus the measured SCC is obviously, at least partly, of non-sodium origin.

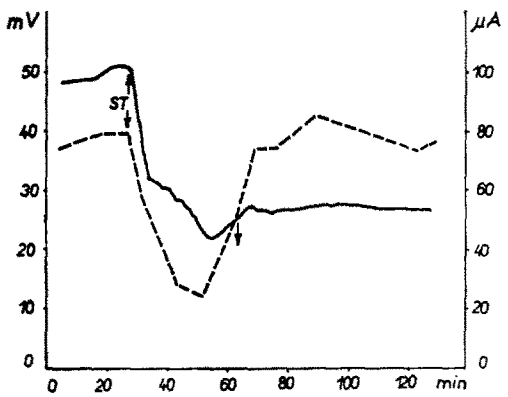


FIG. 1. ST (2000 HU/ml)-action on PD, full line and on intermittently measured SCC, dashed line. ST was 35 min in contact with the inside of the frog skin. Ringer's solution inside and 20 mM NaCl outside.

TABLE 1. SODIUM FLUXES IN μequiv. MEASURED WITH ISOTOPES $^{22}Na$ AND $^{24}Na$ AND THOSE CALCULATED FROM SIMULTANEOUSLY MEASURED SHORT CIRCUIT CURRENT

| | Estimation in μequiv. | | | |
|---|---|---|---|---|
| | Isotopic | | | Electric |
| Treatment | Influx | Outflux | Net flux | Net flux |
| Normal skin | 0·65 | −0·12 | 0·53 | 0·57 |
| ST-treated | 0·675 | −0·86 | −0·185 | 0·965 |
| ST-treated | 0·96 | −1·33 | −0·37 | 1·45 |

The same electric current could be caused either by transport of cations from outside to inside or by transport of anions from inside to outside. Net flux of $Cl^-$ along its concentration gradient could electrically well fit the presumptions. In such case measured PD and SCC would not much depend on cation species present in the outside solution as it was observed in our experiments.

To elucidate the role of chlorides we replaced Ringer's-chloride solution by Ringer's-sulphate one on the inside of the skin. Sulphates are much less permeable and the outward directed transport of anions could be prevented to participate with the cation transport directed inward and thus anions do not modify SCC. In these experiments the above described increase of SCC later after ST administration did not occur. The outside replacement of $Na^+$ by $K^+$ was in this case followed by a depression of PD and SCC too (Fig. 2). Then, $Cl^-$ transport on ST treated skin is a source of SCC increment. The addition of $4 \cdot 10^{-3}$ M KCN outside rapidly abolishes both PD and SCC of $Cl^-$ transporting skin. Therefore $Cl^-$ movement seems not to be a purely passive process.

Concluding, ST blocks the active transport of $Na^+$ first. The $Na^+$ transport is mediated by $Mg^{++}$

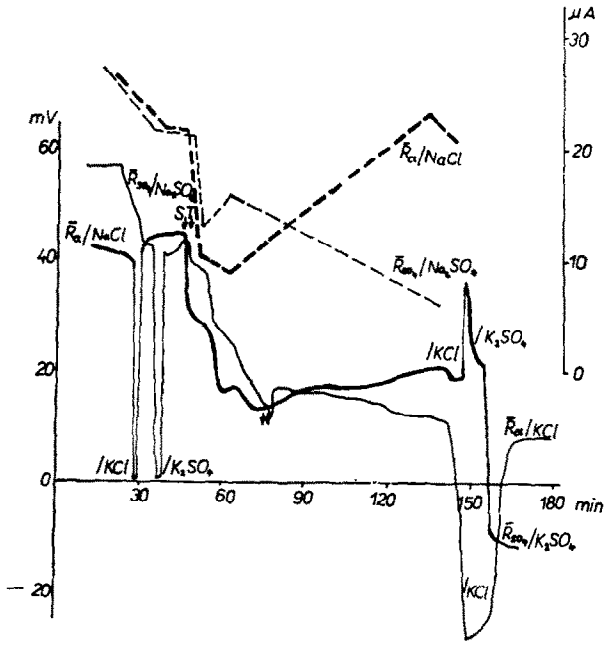


FIG. 2. ST (2000 HU/ml) action on PD (full lines) and on intermittently measured SCC (dashed lines) in Ringer's-chloride solution, $\bar{R}Cl$ (thick full and dashed line respectively) and in Ringer's-sulphate, $\bar{R}SO_4$ (thin full and dashed lines respectively). KCl and $K_2SO_4$ replaced outside NaCl and $Na_2SO_4$ Fractions indicate solutions as present: inside/outside.

$(Na^+ + K^+)$ activated ATPase[6] and this membrane bound enzyme might be inhibited by the toxin. This suggestion is strongly supported by the finding of Novák *et al.*[7] who described a brief stimulation followed by irreversible inhibition of $Mg^{++}(Na^+ + K^+)$ activated ATPase from liver mitochondria.

The impairment of active $Na^+$ transport found in the frog skin model is in agreement with the analogous finding of Rahal *et al.*[8] who treated toad bladder with ST. However, they observed the irreversible loss of PD and SCC only. The different organ and different concentrations of bathing media might be the explanation for our finding of delayed restoration of SCC. The difference of toad bladder and frog skin has been found also with the adrenaline which induces $Cl^-$ flux in frog skin only.[9] The restoration of SCC in our experiments is due to induced $Cl^-$ flux through the membrane. As permselectivity is considered a membrane function[5] permeability changes caused by ST could be located on the cell membrane. In any case this adds to our knowledge about ST activities on cells.

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