58. The Effects of Ouabain on Active Na Transport through Frog Skin

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Since Schatzmann's observation on Na and K transports through red cell membrane, cardiac glycosides have been recognized as the selective inhibitors of active transport of Na and K ions through several kinds of biological membranes. Recently Koefoed-Johnsen and Whitney and Widdas communicated briefly the inhibitory action of ouabain on short-circuit current of the isolated frog skin. Kirschner also described the inhibitory action of strophanthin on Na influx in this material. Present study was made to elucidate the mechanism by which a cardiac glycoside inhibits the active Na transport through the frog skin.

The experiments were carried out on the isolated abdominal skin of Rana nigromaculata. It is placed as a membrane separating the Ringer's solution in two compartments. Short-circuit current, electrical conductance, and the Na outflux by the use of $^{24}$Na, were measured simultaneously on the same preparation. Throughout the experiments the potential difference across the skin was maintained equal to zero by supplying an external emf. The essential features of the experimental set-up are similar to those of Ussing and Zerahn and Linderholm. The room temperature was kept at 20±1°C.

After sufficiently equilibrating the material for the medium, ouabain in various concentrations was added to the corium side or to both sides of the frog skin membrane. The short-circuit current diminished steadily. The repeated washings with ouabain-free Ringer's solutions, as indicated by Fig. 1, revealed that there was only a little if any reversibility in the inhibitory effect of ouabain. Without washing, the short-circuit current continued to decrease never reaching a new equilibrium level until it ran down to zero. The effect of the drug in lower concentrations than $2 \times 10^{-7} \text{M}$ was small and masked by the spontaneous drift of the current. This result was quite different from the effect of 2,4-DNP which was readily reversible under the same condition.

The result agrees well with the finding of Caldwell and Keynes on the irreversibility of the inhibitory action of ouabain in giant axons. As regards the cardiac glycosides other than ouabain, the inhibitory actions on the active ion transports have been reported by several investigators to be reversible on several materials. Ouabain therefore
seems to be unique in the incomplete reversibility of its inhibitory action on the active ion transport.

In potassium rich media the effects of ouabain on the short-circuit current as well as on the other parameters were reduced. This fact is in accordance with the observations by Whitney and Widdas,\textsuperscript{10} Glynn,\textsuperscript{3}

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  \item Fig. 1. Reversibility of the effect of ouabain on the short-circuit current of the frog skin. The black arrows indicate the additions of ouabain to both sides (upper records) and to the corium side (lower records) of the Ringer's solutions to give the final concentrations indicated. The white arrows indicate the washings with the normal Ringer's solutions.
  \item Fig. 2. The effectiveness of ouabain on the short-circuit current in potassium rich-media and Na deficient media. Abscissa: the time for 50% inhibition of the current. Ordinate: reciprocal of the concentration of ouabain
  \item Fig. 3. The effects of ouabain on the Na transport through a frog skin. The arrow indicates the addition of ouabain to both sides of the Ringer's solutions to give the final concentration of $1.23 \times 10^{-6}$ M. I: the short-circuit current in $\mu$A/cm$^2$. Mo: the Na outflux in $\mu$A/cm$^2$. G: the electrical conductance in mmho/cm$^2$. $E_{Na}$: the calculated active Na transport potential in mV. $G_{Na}$: the calculated Na conductance in mmho/cm$^2$
\end{itemize}
and Solomon and Gill. Whereas in sodium deficient media the effectiveness of ouabain on the short-circuit current was the same as in the normal Ringer’s solution. Fig. 2 summarizes the result of the experiments.

As will be seen from Fig. 3, after addition of the drug, electrical conductance, the calculated values of the active Na transport potential \( E_{Na} \), and Na conductance \( G_{Na} \) diminished hand in hand. Above the concentration of \( 1 \times 10^{-6} \) M, a temporary elevation of the electrical conductance often occurred. On the other hand, the increase of the Na outflux was observed even when the electrical conductance decreased. This observation seems to contradict with that of Glynn’s on K outflux through the red cell membrane. At present the nature of this rise in the Na outflux accompanied by the reduction of the electrical conductance is difficult to interpret and awaits for further analysis.

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References