INHIBITION OF THE SODIUM PUMP OF THE FROG'S SKIN BY OUABAGENIN AND STROPHANTHINS K AND G

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Ouabagenin in concentrations below $7 \cdot 10^{-6}$ mole/liter, unlike strophanthin K and ouabain, which completely inactivate the sodium pump of the frog's skin, initially depresses the sodium flux slightly but later has no effect on sodium transport. The presence of sugar in the molecule of the glycoside potentiates its action, evidently by facilitating strong binding with (Na^+, K^+)-ATPase.

Cardiac glycosides and their aglycones have not only a cardiotonic, but also a natriuretic action, and in both cases the dominant factor is inhibition of the sodium pump [3, 5, 6]. Activity of the glycosides is considerably higher than that of the aglycones [1, 10].

To assess the role of the sugar component in the specific action of glycosides it was decided to investigate the kinetics of action of the glycoside and aglycone on sodium pump. Frog's skin was chosen as the test object, for unlike the kidney [8], sodium transport in it is completely abolished by strophanthin [4].

EXPERIMENTAL METHOD

Active sodium transport through the abdominal skin of male winter frogs (Rana temporaria) was studied by measuring the short-circuited current [2]. All the experiments were carried out in constant temperature vessels at 25°C. Preparations of strophanthin G (ouabain) and ouabagenin were provided by the firm Sandoz A. G. (Switzerland), and strophanthin K was obtained from the Factory of the Pharmaceutical Chemical Research Institute in Khar'kov; glycosides and aglycones were added to Ringer's solution bathing the inner surface of the frog's skin.

EXPERIMENTAL RESULTS AND DISCUSSION

Strophanthin K, ouagain, and ouabagenin can completely abolish active sodium transport in the frog's skin (Fig. 1), but the dose of the glycoside required to do this is much smaller than that of the aglycone. However, when concentrations of the drugs depressing sodium transport by 50% were used for about 30 min (Fig. 1, experiments Nos. 2 and 4), qualitative difference was clearly seen between the actions of the glycosides and aglycone. Strophanthin K and ouabain gradually but completely inactivated the sodium pump, whereas ouabagenin initially reduced the sodium transport, but thereafter the operation of the pump became stabilized. This difference in the action of the preparations is also reflected in the relationship between the concentration of the drug and the time during which it reduces sodium transport by 50% (Fig. 2). Both strophanthin K and ouabain are not only more powerful inhibitors of sodium transport, but in all concentrations tested they also produced a progressive inhibition of the sodium pump, the effect being clearly dependent upon the dose. Ouabagenin, by contrast, in concentrations below $7 \cdot 10^{-6}$ mole/liter, initially produced a small decrease in the flux, but the process thereafter became stabilized.

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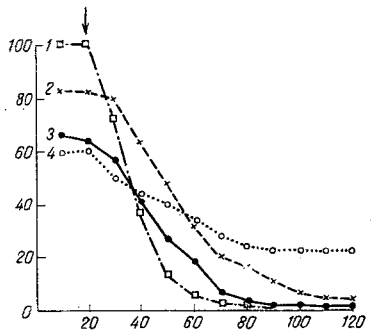

Fig. 1

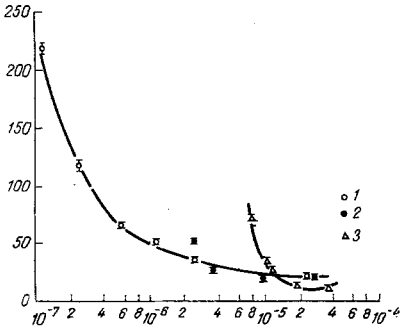

Fig. 2

Fig. 1. Dynamics of action of cardiac glycosides and aglycone on sodium transport in frog's skin. Arrow indicates addition of ouabagenin in concentrations of $3.8 \cdot 10^{-5}$ M (1) and $1 \cdot 10^{-5}$ M (4), strophanthin $2.36 \cdot 10^{-6}$ M (2), and ouabain $3.3 \cdot 10^{-6}$ M (3). Ordinate, short-circuited current (in $\mu A/cm^2$); abscissa, time (in min).

Fig. 2. Time taken to reduce sodium transport by 50% as a function of logarithm of concentration of drug. Ordinate, time (in min); abscissa, log of concentration of strophanthin K (1), ouabain (2), and ouabagenin (3). Each point represents mean result of 6-22 experiments.

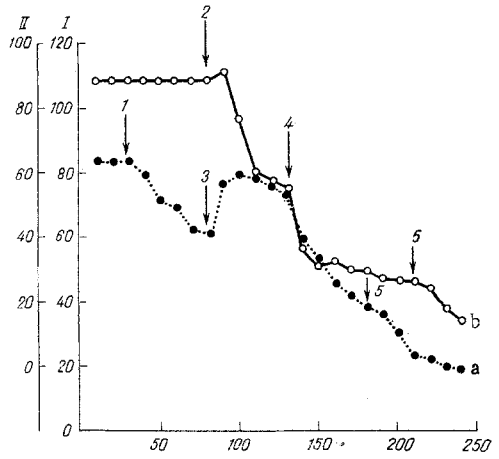

Fig. 3. Effect of ouabagenin on sodium transport: 1) addition of ouabagenin to skin a in dose of $7.6 \cdot 10^{-6}$ mole/liter; 2) solution with ouabagenin from skin a transferred to inner surface of skin b; 3) ouabagenin rinsed out with Ringer's solution; 4) $7.6 \cdot 10^{-6}$ mole/liter ouabagenin added to skin a and b; 5) a further $7.6 \cdot 10^{-6}$ mole/liter ouabagenin added to skin. Ordinate, short-circuited current in $\mu A/cm^2$ (I for preparation of skin a, II for skin b); abscissa, time (in min).

The feature of the action of ouabagenin mentioned above could occur because the aglycone, unlike the glycoside, forms a dissociating complex with the enzyme, and in concentrations below $7 \cdot 10^{-6}$ mole/liter it is impossible to obtain 50% reduction in sodium transport, or the aglycone is firmly bound with the various components of the frog's skin, and for this reason it disappears quickly from the Ringer's solution. To analyze this alternative experimentally experiments were carried out on 10 membranes by the following scheme. Ouabagenin was added to half of the objects, and after depression of the sodium transport, when the process was stabilized, the Ringer's solution with ouabagenin in contact with the inner surface of the skin was transferred to a fresh preparation of skin, while the skin exposed to its action was rinsed with Ringer's solution. The results given in Fig. 3 show clearly that the Ringer's solution with ouabagenin reduced sodium transport in the fresh specimen by the same extent as in the original object and, consequently, the concentration of the inhibitor in solution is not significantly reduced after incubation of skin in it. On the other hand, restoration of the sodium flux after removal of the inhibitor by rinsing demonstrates the instability of its binding with the enzyme. Conversely, after removal of the strophanthin K by rinsing and, as experiments on the urinary bladder of the frog Rana catesbiana have shown, after removal of ouabain, added in a concentration of $1 \cdot 10^{-7}$-$1 \cdot 10^{-4}$ mole/liter by rinsing the sodium transport is not restored [7]. The results thus demonstrate that the characteristic kinetics and the higher activity of strophanthins K and G than of ouabagenin are due to the presence of sugar in the molecule of the glycoside, facilitating its firm bonding, probably with the molecule of (Na^+, K^+)-ATPase. This enzyme is the chief point of action of the cardiac glycosides in the system of active sodium transport [9].

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