High and low affinity ouabain binding sites in guinea-pig atria

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We have previously reported that doses of ouabain between 1 nM and 10 nM stimulate the Na pump and evoke a positive inotropic effect in guinea-pig atria, whereas doses higher than 10 nM inhibit the Na pump (Ghysel-Burton & Godfraind, 1975).

We have also reported that a specific ouabain binding represented by a Langmuir binding curve occurred likely on the sites responsible for the inhibition (Godfraind & Lesne, 1972; Ghysel-Burton & Godfraind, 1975).

The purpose of the present experiments was to examine whether these stimulatory and inhibitory effects of ouabain were related to one mode of specific binding.

Experiments were performed on isolated guinea-pig atria (resting tension 500 mg) stimulated at a rate of 3.3 Hz and bathed in Tyrode solution at 30°C (mM: NaCl 137; KCl 2.7 either 6 or 12, $CaCl_2$ 1.82, $MgCl_2$ 0.105, NaH_2PO_4 0.417, $NaHCO_3$ 11.9, glucose 5.5) equilibrated with a mixture of 95% O_2/5% CO_2. [^{3}H]-ouabain was added together with [^{14}C]-inulin in the incubation fluid. This procedure allowed to correct tissue ouabain uptake for extracellular ouabain content.

For 6 mM KCl, within the range of ouabain concentrations comprised between 30 and 10^3 nM, the cellular ouabain uptake showed both a linear and a saturable component and was found to fit the equation:

$$U = a\,Cm + \frac{b\,Cm}{Cm + Kb} \qquad (1)$$

where U is the tissue concentration corrected for cardiac glycoside content of inulin space, Cm is the glycoside concentration in the medium, a is the proportionality constant for the linear non-saturable uptake, b is the capacity and Kb is the equilibrium constant for the saturable binding sites (Godfraind & Lesne, 1972). Values of the constants were as follows: a (1/kg wet weight) 0.319, b (n mol/kg wet weight) 372, Kb (nM) 389.

For ouabain concentrations between 1 nM and 10 nM, the observed ouabain tissue content was higher than that expected from equation (1) using the constants given above. After subtracting the non-saturable uptake, aCm, from the observed contents, the saturable uptake showed two components, one at concentrations between 1 and 10 nM and the other at higher concentrations.

This indicates the existence of a high and of a low affinity uptake process. The high affinity process was represented by a S shaped curve with a Hill coefficient near 2; the concentration producing a 50 per cent saturation was equal to 2.5 nM. The capacity of this binding was only 3 per cent of the capacity of the low affinity binding represented by equation (1).

Both high and low affinity bindings were sensitive to change in KCl concentration. For 2.7 mM KCl, the two bindings were displaced to the left. They were displaced to the right for 12 mM KCl.

The sensitivity of the two bindings to extracellular KCl indicates that both might occur on Na pumping sites.

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References

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