Stimulation and inhibition by ouabain of the sodium pump in guinea-pig atria

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Studies on isolated human heart preparations have shown that, within the range of free therapeutic concentrations ($\pm 3 \times 10^{-9}$ M), digitoxin did not inhibit the sodium pump but stimulated ^{42}K uptake (Godfraind, 1973). It has also been reported that low ouabain concentrations (10^{-9} M) stimulated ^{42}K pumping in guinea-pig atria and produced a supersensitivity to catecholamines (Godfraind & Godfraind-De Becker, 1965; Godfraind & Lesne, 1972). The present experiments, performed on electrically stimulated left guinea-pig atria, were designed in order to see whether the actions of therapeutic concentrations might be related to the binding of ouabain to the cardiac glycoside receptors responsible for the inhibition of the sodium pump.

Isolated left guinea-pig atria were bathed at 30° C in Tyrode solution (mM : NaCl 137, KCl 6 instead of 2.7, $CaCl_2$ 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. The atria were electrically stimulated at a rate of 3.3 Hz. Their contractility was recorded by means of isometric levers (resting tension ± 500 mg). They were stimulated by cumulative increment of isoprenaline up to 10^{-5} M. After 30 min resting period, ouabain, at concentrations of between 10^{-9} and 10^{-4} M, was added for three hours. At the end of the experiment, the atria were mineralized and the ionic content was determined by atomic absorption spectroscopy. The binding of [^{3}H]-ouabain was estimated as previously reported (Godfraind & Lesne, 1972).

Low concentrations of ouabain (10^{-9} to 10^{-8} M) stimulated the sodium pump as shown by the increase of the K content associated with an equivalent decrease of the Na content (mmol/kg wet wt; controls, (15), Na : 74.8 ± 1; K: 63.9 ± 0.9; atria incubated with ouabain 3×10^{-9} M (8) Na: 70.1 ± 1.4, K: 68.4 ± 1.3; $P = 0.02$).

Higher concentrations inhibited the pump, a dose-dependent Ca gain associated with a Mg loss was noticed when the inhibition reached 30%.

The positive inotropic effect of ouabain occurred for concentrations stimulating the Na pump, it increased with the inhibition of the pump. The maximum increase in systolic tension was of 250%, the dose producing 50% of the maximum was 3×10^{-7} M. For ouabain concentrations lower than 3×10^{-7} M, the increase in systolic tension was maintained over the 3 h of the observation period. At higher concentrations, the increase in systolic tension reached an optimum and decreased with the increase of the diastolic tension.

Confirming earlier results, [^{3}H]-ouabain uptake showed a linear and a saturable component (Godfraind & Lesne, 1972). The latter was a Langmuir binding curve with a K_e of 4×10^{-7} M and approaching complete saturation at 3.4×10^{11} molecules of ouabain per mg wet wt, it intersected the dose-effect curve relating ouabain-sensitive K content to ouabain concentration near 50% of the maximum. This provides evidence that the saturable binding sites and the pumping sites are the same. A further evidence was provided by their $(K)_o$ dependency: for a reduction of $(K)_o$ from 6 mM to 2.7 mM the Langmuir binding curve was displaced to the left with a K_e of 1.9×10^{-7} M and a complete saturation at 1.3×10^{11} molecules of ouabain per mg wet wt; the dose effect curve for the action of ouabain on the pump was displaced to the same extent; the dose producing 50% of the maximum inotropic effect was reduced to 4×10^{-8} M. Furthermore, the maximum increase in systolic tension was only of 80%, as if the inotropic effect was related to the number of ouabain molecules bound.

It has been reported that the contractility of the atria was dependent on $(Na)_i$ (Glitsch, Reuter & Scholz, 1970). The systolic tension and the Na content were respectively 34% and 10% lower for $(K)_o = 6$ mM than for $(K)_o = 2.7$ mM.

This indicates that the contractility was reduced by $(K)_o$ stimulation of the sodium pump.

As the pump stimulation by ouabain is associated with an increase in systolic tension, the interaction of the glycoside with the receptor probably involves supplementary changes.

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