The Effect of Ouabain on the Alga *Hydrodictyon reticulatum*

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The action of the cardiac glycoside ouabain on animal cells was thoroughly studied and its characteristics are well known (see Weatherall 1962): the cells gain sodium and lose potassium, the cell membrane potential decaying. This is commonly interpreted as a result of a specific sodium-potassium transport inhibition. The effects of ouabain were also looked for on giant cells of Characeae; some of the findings appear to be analogous to those registered on animal cells, others, however, appear to be different. Thus the influx of potassium in the internodal cells of *Nitella translucens* was decreased by 5×10^{-5} M ouabain, but after long treatments with the glycoside there was a tendency for potassium influx to increase in some cells (MacRobbie 1962). Spanswick and Williams (1964) found no effects of ouabain on the membrane potentials across both plasmalemma and tonoplast with the same species.

In the present paper the effects of rather high concentrations (from 10^{-4} to 10^{-3} M) of ouabain after prolonged treatments (one to three days) on a different alga, the Chlorophycean *Hydrodictyon reticulatum*, are described. The used concentration of the inhibitor was chosen as it is known to be sufficient for a total inhibition of the active sodium transport in animal tissue (Whittam and Willis 1963).

Materials and methods

Experiments were carried out using networks of the alga *Hydrodictyon reticulatum* inoculated and grown in 250 ml borosilicate flasks at room temperature in artificial pond water of the following composition (concentration, mM): 0.5 NaCl, 0.368 KH_2PO_4 , 0.287 K_2HPO_4 , 0.2 CaCl_2 , 0.05 MgSO_4 , 0.55 KNO_3 , 0.333 Fe-citrate; to one liter of this medium 1 ml of a stock solution of trace elements (0.2 FeSO_4 , 0.2 H_3BO_3 , 0.05 MnSO_4 , 0.2 ZnSO_4 , 0.1 CuSO_4 , 0.01/ $\text{NH}_4/6\text{Mo}_7\text{O}_{24}$, 0.1 CoCl_2) was added, occasionally together with 5 ml of a soil decoction. Approximately five weeks after the inoculation the algae were transferred to artificial pond water of the same composition (without trace elements) and the experiments were carried out, the experimental media being now replaced by fresh salines every 24 hours. The potential difference between the outside and inside of the cells was measured using microelectrodes of borosilicate glass, with the tip diameter approximately 1μ , filled with 3M KCl and connected by means of calomel electrodes to a Pye millivoltmeter. The water content of the cells was determined from the loss of weight of the cells by drying: the networks were blotted by a gentle pressure between two sheets of filter paper, then weighed, dried at 95°C overnight and reweighed. The cell water was expressed in kg per kg of dry solid (DS). The determination of ions was carried out after a five day extraction of dry solids in 0.2M HNO_3 . Sodium and potassium were determined on the EEL flame photometer, chloride by a potentiometric microtitration (Ramsay *et al.* 1965) and calcium spectrophotometrically with sodium naphthalohydroxamate as reagent (Höfer 1963). Cell ions were expressed either in mmoles/kg DS or as apparent concentrations (mmoles/kg cell water).

Results

A highly significant increase in the water content was observed when the *Hydrodictyon reticulatum* cells were kept from one to three days in the artificial pond water with ouabain concentration ranging from 10^{-4} to 10^{-3} M. The results on the swelling of the cells are summarized in Table 1. It will be seen from Experiment No. 3 that the extent of the swelling in the given time limit is still dependent on the concentration of the inhibitor.

Table 1. The effect of ouabain on the water content of *Hydrodictyon reticulatum*

Exp. No.	Ouabain concentration	Inhibitor applied for	Water content in kg/kg DS		Significance level
			Controls	Ouabain treated	
1	10^{-4} M	3 days	9.70 ± 0.42 (n=10)	12.28 ± 0.64 (n=10)	$p < 0.005$
2	5×10^{-4} M	1 day	12.51 ± 0.21 (n=7)	14.51 ± 0.46 (n=8)	$p < 0.005$
3	10^{-4} M	1 day	12.53 ± 0.39 (n=12)	15.27 ± 0.59 (n=12)	$p < 0.005$
	10^{-3} M			17.58 ± 0.44 (n=12)	$p < 0.001$
4	10^{-4} M	2 days	10.13 ± 0.22 (n=8)	11.70 ± 0.46 (n=8)	$p < 0.01$
5	10^{-4} M	3 days	14.35 ± 0.24 (n=11)	16.52 ± 0.27 (n=11)	$p < 0.001$

When kept 3 days in 10^{-4} M solution of ouabain in artificial pond water the cells of *Hydrodictyon reticulatum* showed no significant depolarization when compared with analogously treated controls: the means $\pm$ S.E. were 78.9 ± 4.1 mV (n=7) and 71.7 ± 3.7 mV (n=7) for controls and ouabain series, respectively. No attempts were made to find out or to correct for the (presumably low) potential difference across the tonoplast surrounding the central vacuole. A highly significant swelling took place in this experiment; the corresponding values for the water content are given in Table 1., Exp. No. 1.

The amount of potassium, sodium, calcium and chloride ions was determined both in normal cells and cells treated for 3 days with 10^{-4} M ouabain. The results are shown in Table 2. A highly significant net increase may be observed both in the potassium as well as chloride ion content (175 ± 44 mmoles K^+ and 157 ± 32 mmoles Cl^- per kg DS); on the other hand, the amount of sodium and calcium ions apparently did not change. Simultaneously with the changes of ion contents a pronounced swelling (presented in Exp. No. 5, Table 1.) took place. On computing the apparent concentrations of potassium and chloride ions it will be seen that, irrespective of a significant net uptake of these ions, their concentrations

remain approximately constant, viz. 139.1 ± 3.0 and 131.4 ± 3.0 mmoles K^+ /kg cell water and 45.5 ± 1.5 and 48.9 ± 1.8 mmoles Cl^- /kg cell water in the controls and ouabain series, respectively.

Table 2. The effect of ouabain (10^{-4} M, 3 days) on the content of ions in *Hydrodictyon reticulatum*

Ion	Controls mmoles/kg DS	Ouabain treated mmoles/kg DS	Significance
K	1996 ± 28 (n=11)	2171 ± 34 (n=11)	$p < 0.001$
Na	65.2 ± 2.1 (n=9)	63.8 ± 3.9 (n=9)	
Ca	46.4 ± 3.8 (n=9)	42.1 ± 3.4 (n=9)	
Cl	652 ± 19 (n=11)	809 ± 26 (n=11)	

Discussion

Ouabain thus brings about a swelling of the cells of *Hydrodictyon reticulatum*, associated with a gain of potassium chloride, the sodium and calcium content as well as the potential difference across the cell membrane remaining approximately constant. According to our preliminary results (to be published) there appears to be also in *Hydrodictyon reticulatum* an active sodium pump, operating in the outward direction (together with a chloride pump directed inwardly). The present paper shows that the effect of ouabain in concentration range 10^{-4} – 10^{-3} M is rather unlike the familiar inhibition of the active sodium extrusion by the animal cells. The considerable swelling of the *Hydrodictyon reticulatum* cells caused by ouabain appears to be due to a net transport of potassium chloride solution of a concentration similar to that inside of the cells. This sort of action seems to be of a different nature than an inhibition of an ion pump. It rather appears as if a part of the turgor pressure exerted by the cell wall were released or some other mechanism extruding water from the cells were inhibited by ouabain, so that water is then driven osmotically into the cells. The intracellular potassium chloride would be diluted by this process and its entry at a constant membrane potential enhanced, whereas increase in the intracellular sodium and calcium seems to be prevented by an impermeability or outwardly directed pumps.

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References

- Höfer, M. 1963. *Experientia* 19: 367.
- MacRobbie, E. A. C. 1962. *J. Gen. Physiol.* 45: 861.
- Ramsay, J. A., Brown, R. H. J. and Croghan, P. C. 1955. *J. Exp. Biol.* 32: 822.
- Spanswick, R. M. and Williams, E. J. 1964. *J. Exp. Bot.* 15: 193.
- Weatherall, M. 1962. In Munger, J. L. and Reuck, A. V. S. 1962. *Enzymes and Drug Action*. J. and A. Churchill, London.
- Whittam, R. and Willis, J. S. 1963. *J. Physiol.* 168: 158.
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