Effects of Amiloride on the Transport of Sodium and Other Ions in the Alga *Hydrodictyon reticulatum*

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Abstract. The diuretic amiloride, an almost specific inhibitor of sodium transport in animal cells and tissues, appears to produce a number of effects in the alga *Hydrodictyon reticulatum*. At 1 mmol/l concentration it markedly reduces the influx of sodium ions (but not their active outflux), the influxes of potassium, chloride as well as of bicarbonate ions, and causes a profound decrease in the plasmalemma membrane potential. This plurality of inhibitory effects suggests that individual transport processes in the alga are mutually coupled.

Key words: Amiloride — Ion transport — Alga *Hydrodictyon reticulatum*

Introduction

The well-known diuretic amiloride (3,5-diamino-6-chloropyrazinoylguanidine hydrochloride) has been fittingly denoted" amolecular probe of sodium transport in tissues and cells": in micromolar concentrations it blocks selective sodium channels in animal cells, whereas at higher concentrations (mmol/l) it inhibits the electroneutral exchange of sodium cations for protons (Benos 1982). There seems to be single exception in animal cells to the sodium-specific action of amiloride: it also inhibits bicarbonate absorption by rabbit cortical collecting tubules in vivo, independently of its action on the sodium transport (McKinney and Burg 1978).

Sodium extrusion from the cells of the alga *Hydrodictyon reticulatum* is obviously an active process; however, it is different from the sodium pump of animal cells, being insensitive to ouabain (Janáček and Rybová 1966; Rybová et al. 1972). The present work was an attempt to examine the sensitivy of both the sodium transport and other transport processes in the alga to amiloride.

Materials and Methods

Mature cells of the alga *Hydrodictyon reticulatum* were used in the experiments. The cultivation procedure consisted in transferring small nets of algal cells (a few days after they had been released from maternal cells) into sterilized 250 ml Erlenmayer flasks containing 80 ml of the cultivation medium (mmol l): $NaCl$ — 0.50, KH_2PO_4 — 0.367, K_2HPO_4 — 0.287, $CaCl_2$ — 0.20, $MgSO_4$ — 0.05, KNO_3 — 0.55 and Fe-citrate — 0.05. To each litre of this solution, 5 ml of soil decoction and 1 ml of solution with trace elements (mmol l: $FeSO_4$ — 0.20, $ZnSO_4$ — 0.20, $CuSO_4$ — 0.10, $MnSO_4$ — 0.05, $CoCl_2$ — 0.10, $(NH_4)_6Mo_7O_{24}$ — 0.01, H_3BO_3 — 0.20) were added. The algae were grown for three weeks at room temperature and normal day-and-night pattern of illumination with fluorescent tubes.

Unless otherwise stated, the experiments of the present study were carried out in artifical pond water containing $NaCl$ 1 mmol l, KCl 0.1 mmol l and $CaCl_2$ 0.1 mmol l. The algae were equilibrated in this medium overnight before the experiments.

Intracellular microelectrodes with a tip diameter below 1 µm were prepared from micropipettes drawn on an electromagnetic puller from borosilicate capillaries with inner fibres, which made direct filling with 3 mol l KCl easy. The tip potentials and resistances of the microelectrodes, measured both before and after the impalement, were below 5 mV and about 10 MΩ, respectively.

Wet weights of the cells were obtained by weighing thoroughly blotted cells on a torsion balance, and dry solids by weighing on a semimicrobalance the same samples dried in Teflon vessels overnight at 95 °C. For radioactivity and flame photometry measurements, dry solids were extracted for four days in 0.01 mol l sulphuric acid. The radioactivity of ^{22}Na and ^{42}K was measured using a crystal scintillator, that of ^{36}Cl with a liquid scintillator. An EEL flame photometer was used to determine the sodium content of the algae.

Alkalinization of the medium by illuminated algae was measured in suspensions attached directly to the surface of a glass electrode by a nylon net (Rybová et al. 1980). The intensity of illumination by incandescent bulb was about 3500 lx.

Amiloride used in the experiments was MIDAMOR (Léčiva, Praha, produced in collaboration with Merck and Co., Inc. Rahway, N.J., U.S.A.).

The significance of the differences observed was assessed using Student's t-test.

Results

The most conspicuous effect of amiloride on phenomena related to transport of ions in the alga *Hydrodictyon reticulatum* involved that on the intracellular electrical potential measured with a conventional glass microelectrode (borosilicate microbridge filled with KCl). Amiloride (1 mmol/l) always decreased the electrical potential measured against the external medium with the tip of the microelectrode in the vacuole. In 11 experiments the original potential level of 111 ± 6 mV was reduced during 10 ± 1.4 minutes to the value of 48 ± 8 mV ($p < 0.001$). The rather large scatter of original membrane potential values (from 95 mV to 154 mV) corresponds to genuine variability of individual samples of algal cells.

In view of the ability of amiloride to inhibit transport of sodium ions in animals cells, analogous phenomena were the next to be studied in the alga.

Fig. 1 shows the efflux of labelled sodium from the cells of the alga *Hydrodictyon reticulatum*, equilibrated with ^{22}Na for four days and, at time zero, transferred into non-radioactive medium. The experiment was performed in the light. There seems to be very little (if any) reduction of the sodium efflux with 1 mmol/l amiloride.
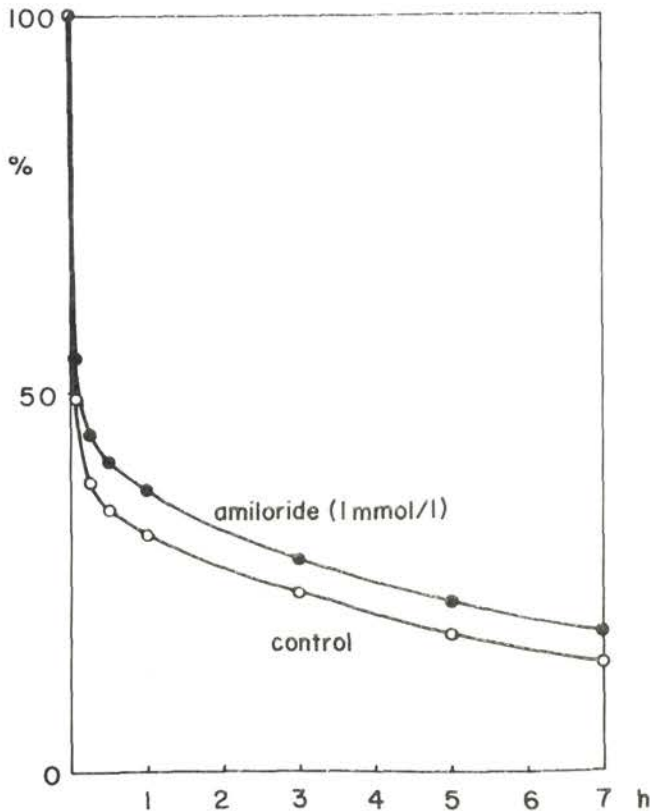

Fig. 1. Efflux of ^{22}Na-labelled sodium from the alga *Hydrodictyon reticulatum* into nonactive medium. Ordinate: per cent of the initial activity.

On the other hand, the effect of 1 mmol/l amiloride on the inflow of sodium both from 0.1 and 1.0 mmol/l sodium is indisputable; as shown in Table 1 the inflow of labelled sodium during three hours is reduced to about one half. In analogous series of experiments, amiloride at a concentration of 0.1 mmol/l also reduced the inflow from 1.0 mmol/l Na$^+$ solution ($p < 0.001$), but only by 18 %; at 0.01 mmol/l it even stimulated the inflow ($p < 0.005$) by 14 %.

The effect of amiloride (1 mmol/l) on the total sodium content of the alga

was in agreement with both its reducing effect on the sodium influx and practical absence of any effect on the sodium efflux: after 3 hours of treatment the apparent sodium concentration in the cell water (1.56 ± 0.07 mmol/l, $n = 10$) was highly significantly lowered as compared with that in untreated cells (3.14 ± 0.14 mmol/l, $n = 10$).

Table 1. Labelled sodium inflow into the alga *Hydrodictyon reticulatum* (mmol/kg wet weight of algal sodium labelled with ^{22}Na after 3 h of incubation) as influenced by amiloride (1 mmol/l).

Na$^+$ concentration in the medium (mmol/l)	controls	amiloride
	light	
0.1	0.170 ± 0.002 [$n = 6$]	0.095 ± 0.003 [$n = 6$]
1.0	1.75 ± 0.04 [$n = 6$]	0.85 ± 0.01 [$n = 6$]
	dark	
0.1	0.132 ± 0.004 [$n = 6$]	0.087 ± 0.003 [$n = 6$]
1.0	1.42 ± 0.03 [$n = 6$]	0.74 ± 0.03 [$n = 6$]
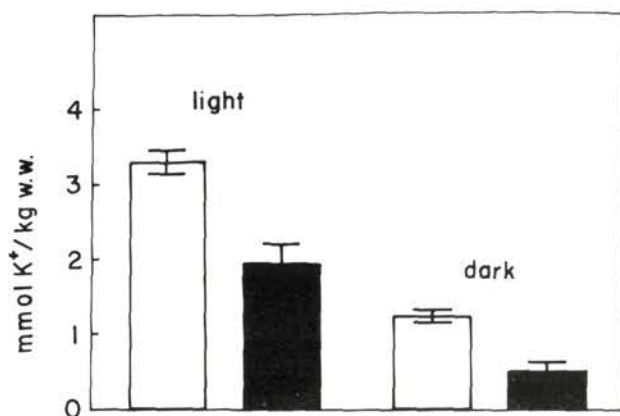
Fig. 2. Effect of amiloride (1 mmol/l) on inflow of ^{42}K-labelled potassium ions measured after 2.5 h of incubation. Averages of 5 measurements $\pm$ S.E.M. are shown. White columns — controls, black columns — amiloride.
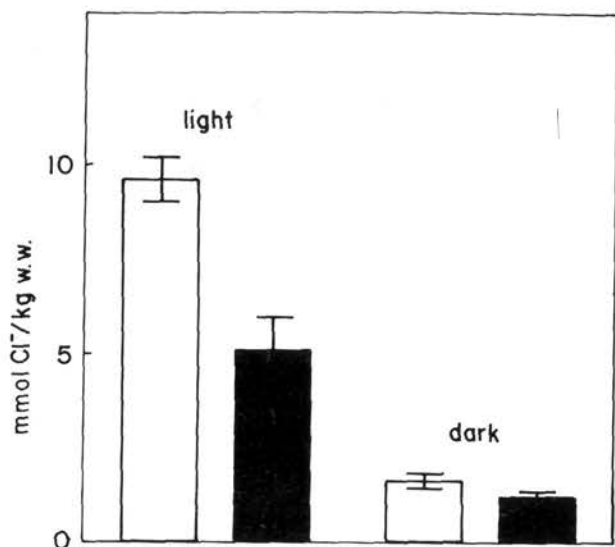
Fig. 3. Effect of amiloride (1 mmol/l) on inflow of ^{36}Cl-labelled chloride ions measured after 5 h of incubation. Averages of 5 measurements ± S.E.M. are shown. White columns — controls, black columns — amiloride.
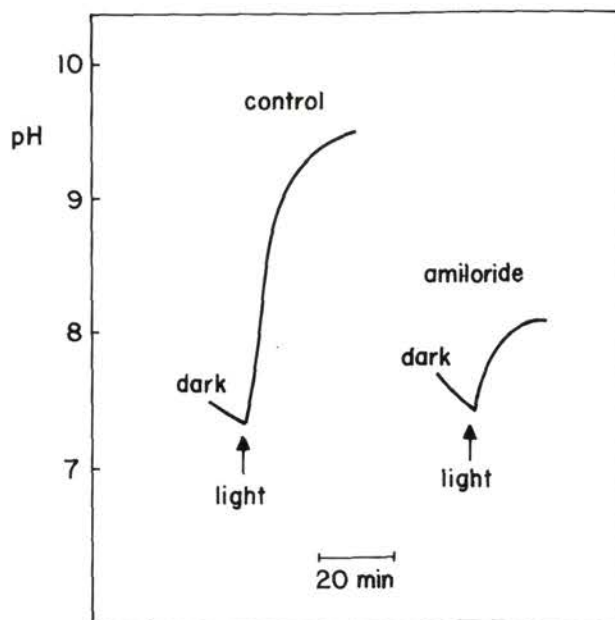

Fig. 4. Effect of amiloride (1 mmol/l) on alkalinization in a suspension of the alga *Hydrodictyon reticulatum*. A typical result chosen out of six analogous experiments.

To examine whether the effects of amiloride in the alga are specifically limited to transport of sodium ions the influence of the drug on the inflows of potassium and chloride ions was determined. Figs. 2 and 3 show that the entry of the two ions is impaired with 1 mmol/l amiloride, both in the light and in the dark. (The fluxes of ions are generally reduced in the dark, as shown by Rybová et al. 1972.) When compared with the controls, the potassium inflow in the light was inhibited to 59 % ($p < 0.001$), the inflow of chloride ions to 53 % ($p < 0.005$); the dark inflows were decreased to 40 % for potassium ($p < 0.001$), but only to 75 % for chlorides (not significant, $0.05 < p < 0.1$). Amiloride had no inhibitory effect on inward transport of chloride anions at a lower, $5 . 10^{-4}$ mol/l concentration, this being in contrast to its effect on sodium ion influx.

Finally, as shown in Fig. 4, also the alkalinization in the suspension of illuminated algae, believed to result from the uptake of bicarbonate anions in exchange for hydroxyl anions (Rybová and Janáček 1982), is inhibited by 1 mmol/l amiloride.

Discussion

A general conclusion from the above results may be that amiloride appears to be a much less selective inhibitor in the alga *Hydrodictyon reticulatum* than it is in animal cells and tissues; while the inhibition of bicarbonate transport in the collecting tubules by amiloride seems to be quite an exception in the animal kingdom, in the alga it impaires the fluxes of sodium, potassium, chloride and bicarbonate. The substantial reduction of membrane potential observed with amiloride might explain the diminished influx of potassium cations as being a result of a decrease of the driving electrical force; however, this cannot serve as an explanation for the diminished influx of the negatively charged chloride anions.

The depolarization of membrane potential itself remains something of a mystery. It cannot be related to the decreased influxes of the sodium and potassium cations, since a decreased inflow of positive charges would result in hyperpolarization. Neither does it seem probable that inhibition of an inwardly directed electrogenic anion pump is responsible for the fall of the membrane potential. The pumping of chloride anion is markedly reduced in the dark or with the inhibitor of the 2nd photosystem (Rybová et al. 1972, see also Fig. 3 of the present paper) and we never observed a comparable depolarization under these two conditions; the membrane potential remains often unaffected. Similarly, the bicarbonate uptake does not appear to be electrogenic, being inhibited by inhibitors of carbonic anhydrase without a change in the membrane potential

(Rybová et al., in preparation). Our preliminary results with diethylstilbestrol, an inhibitor of H^+-ATPase (Vara and Serrano 1982), suggest that inhibition of an outwardly directed electrogenic proton pump may be responsible for the phenomena such as the depolarization with amiloride, described in the present paper. If this is really the system that is primarily attacked by amiloride, the following difference should be noted: while in animal cells 1 mmol/l amiloride inhibits an electroneutral Na^+/H^+ exchange, electrogenic exchange should be blocked by the same drug in the alga. Inhibition of the proton pump may then explain the marked decrease in the chloride inflow in the light provided that the latter transport mechanism is secondarily dependent on the former. It may also be that after amiloride treatment the permeability of the plasmalemma for potassium cations becomes less important as compared with that for chloride anions; this, too, would result in a reduction of the membrane potential. The whole problem is hoped to be a subject to further experimental studies.

Nevertheless, the plurality of the inhibitory effects of amiloride in the alga *Hydrodictyon reticulatum* tempts one to speculate that in this alga various transport processes are rather tightly coupled together in such a way that a single chemical agent, almost specific in animal cells, tends to impair a number of processes at the same time.

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