ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1986, p. 958–959
0066-4804/86/050958-02$02.00/0

Vol. 29, No. 5

Effect of Amiloride on the Intracellular Sodium and Potassium Content of Intact *Streptococcus faecalis* Cells In Vitro

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Received 30 August 1985/Accepted 4 February 1986

Amiloride at millimolar concentrations caused marked changes in the growth-dependent intracellular balance of Na^+ and K^+ in *Streptococcus faecalis*. These results, whether specific to transport processes or resulting from indirect yet unknown mechanisms, constitute the first evidence of an effect of amiloride on bacterial electrolytes.

Amiloride, a diuretic widely used clinically for its natriuretic effect, inhibits the proliferation of animal cells (7, 9, 10). Moreover, we reported that amiloride possesses antibacterial activity (2, 3). Although the drug is known to be an inhibitor of passive sodium influx and is currently used in basic and applied research as a molecular probe in sodium transport investigations (1), the exact mechanism of its growth inhibitory properties is not yet fully clear (8).

Recently, it was reported that amiloride at millimolar concentrations inhibits Na^+,K^+-ATPase in certain animal systems (11); interestingly, a recent discovery showed that *Streptococcus faecalis* possesses a K^+/Na^+ antiporter driven by ATP (6).

Taking into account that our previous results indicated that the growth of *S. faecalis* strains is inhibited by millimolar concentrations of amiloride, we decided that it may be important to investigate the effect of the drug on the sodium and potassium balance of this bacterium.

In view of the fact that the K^+/Na^+ antiporter driven by ATP constitutes a new biological entity in the physiology of *S. faecalis* and that direct investigations on its activity present serious technical problems complicated by risks of experimental artifacts (5), we believed that an important insight into this and related problems concerning electrolyte balance may come from an indirect investigation into the effects of amiloride on the intracellular concentrations of Na^+ and K^+ in intact *S. faecalis* cells. In this report, we present evidence that amiloride can cause marked changes in the intracellular concentrations of sodium and potassium. It remains to be established whether the changes observed are the result of a direct action on transport processes or an indirect effect on a yet unknown mechanism.

Two *S. faecalis* strains were used in this study: ATCC 19433 and a strain from a clinical specimen isolated in our laboratory. Mueller-Hinton broth (E. Merck AG, Darmstadt, Federal Republic of Germany) was used; its composition per liter was 2.0 g of beef infusion, 17.5 g of casein hydrolysate, 1.5 g of starch, 125 mM Na^+, and 2.56 mM K^+; the final autoclaved medium had a pH of 7.6.

Amiloride (*N*-amidino-3,5-diamino-6-chloropyrazinecarboxamide)-hydrochloride was a gift of Merck Sharp & Dohme, Rahway, N.J. The product was dissolved as previously described in detail (2). For the determinations of intracellular Na^+ and K^+, we followed exactly the standardized methodologies described by Zarlengo and Schultz (12). Na^+ and K^+ concentrations were determined by using an atomic absorption spectrophotometer (Perkin-Elmer 305A). After we established the ionic balance of the strains used in this study, amiloride was introduced at various concentrations in stationary-state cells before transfer into new fresh medium. In *S. faecalis*, the intracellular ionic composition varies with the phase of growth (4, 5, 12), i.e., stationary-state cells are depleted of K^+ and enriched with Na^+, and when cells are transferred to fresh medium (growth phase), Na^+ extrusion and K^+ accumulation take place (12).

The intracellular Na^+ and K^+ concentrations in the *S. faecalis* strains tested during the stationary and growth phases are shown in Table 1. The data show that after 18 h of incubation at 37°C the cells were depleted of K^+ and enriched with Na^+ and that when these cells were transferred to fresh broth there was an increase in K^+ concentrations and a decrease in Na^+ concentrations, which are known to be the result of K^+ accumulation and Na^+ extrusion. Amiloride was introduced at various concentrations into stationary-state cells, and the changes in the electrolyte balance following transfer into fresh medium containing amiloride were monitored. The drug had no effect on monovalent cation variation at 1.0 mM; however, higher doses caused marked changes with respect to controls (Fig. 1). Amiloride clearly diminished or abolished the normal increase in the intracellular K^+ concentration which happens in the growth phase, and, interestingly, this effect on K^+ took place at the 2.5 mM drug concentration, which also caused growth inhibition in both the streptococci tested. The

TABLE 1. Intracellular sodium and potassium concentrations in *S. faecalis* cells during the stationary and growth phases

Organism and growth phase	Concn (mM)[a]	
	Na^+	K^+
ATCC 19433		
Stationary phase	170	60
Growth phase	80	200
Clinical isolate		
Stationary phase	205	20
Growth phase	90	120

[a] Each value reported represents the mean of triplicate determinations; standard deviations, not reported, never exceeded 21 for Na^+ and 16 for K^+.

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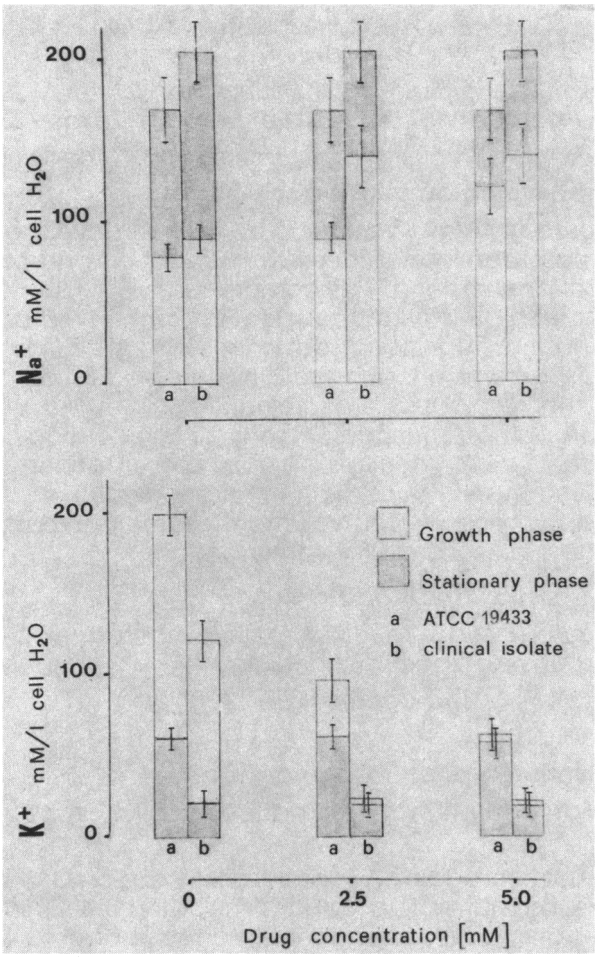

FIG. 1. Changes in intracellular concentrations of sodium and potassium in stationary-phase amiloride-treated cells after transfer into fresh medium. Each result represents the mean of triplicate determinations and the relative standard deviation.

effect of amiloride on the normal decrease in the intracellular content of Na^+ in the growth phase also is shown in Fig. 1. Only strain ATCC 19433 failed to present significant variation at 2.5 mM; otherwise, amiloride-treated cells extruded less Na^+ than did control cultures.

All the experiments were performed in Mueller-Hinton broth, in which *S. faecalis* grows at a slow rate without causing important changes in medium pH. Preliminary experiments seemed to indicate that in nutrient-rich media amiloride causes less-marked changes in monovalent cation concentration. When amiloride-treated cells were suspended in amiloride-free fresh growth medium, they showed a clear replication defect: only after a remarkable delay time (2.5 h) with respect to untreated control cultures did amiloride-treated cells recover their ability to grow. We previously showed that amiloride-treated *S. faecalis* cells remain viable (3), and the fact that upon removal of the drug from the culture medium the cells recovered, after a delay, their ability to divide seems to indicate an effect on a physiological process rather than a generalized toxic effect.

The transport of K^+ and Na^+ by streptococci is a complex, poorly understood area, and newer insights suggest a linkage between Na^+ and K^+ fluxes in *S. faecalis* (6). The evidence suggests that K^+ uptake occurs by two sytems: a proton/K^+ symporter activated by ATP, possibly driven by the proton motive force, and a K^+/Na^+ antiporter driven by ATP (6). It was recently shown that amiloride at millimolar concentrations inhibits Na^+,K^+-ATPase in animal cells (11). In this report, we show that amiloride caused marked changes in both the K^+ and Na^+ content of intact *S. faecalis* cells. Drug concentrations of 2.5 and 5 mM appeared to interfere or block the increase in K^+ concentration which followed the transfer of stationary-state cells into fresh medium. A partial interference was also evidenced with the decrease in Na^+ concentration which proceeded simultaneously. Although the investigation described here cannot give information on the site of action of amiloride, this is of interest. The observation that the effect on K^+ content took place at the same concentration which caused growth inhibition does not demonstrate a causal relationship but certainly stimulates further research. In fact, if, as in animal cells, amiloride at millimolar concentrations acts as a specific inhibitor of K^+/Na^+-linked bacterial fluxes, then specific studies will be of great interest both in transport physiology and for identifying a possible new mechanism through which antibacterial action is realized.

We thank Onofri Adriana (Ufficio di Igiene, USL 12, Ancona) for atomic absorption measurements.

This work was supported by a grant from the Italian Ministero della Sanità, Ricerca Finalizzata Farmacologia Geriatrica sottoprogetto FG4.

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