STUDIES WITH GUANIDINES ON THE MECHANISM OF K^+ TRANSPORT IN YEAST

A. PEÑA

Departamento de Bioquímica, Facultad de Medicina, Ciudad Universitaria, Apartado Postal 70159, Mexico 20, D.F., Mexico

Received 13 April 1973

1. Introduction

A series of effects of guanidines and its substituted derivatives have been reported, ranging from the hypoglycemic effect of synthalin [1] to the inhibitory action of several guanidine derivatives on mitochondrial metabolism [2–4]. More recently, Hille has reported on the affinity of guanidine, among other substances, to the Na^+ channel in nerve [5]. These findings point to a possible relation between the effects of these compounds and their similarity to monovalent cations in biological systems. From data obtained before, we have been inclined to postulate in yeast the existence of a proton pump as the mechanism responsible for the creation of the electrochemical potential necessary to transport K^+ into the cell by means of a specific carrier [6]. Guanidines seemed convenient tools to study further the transport of this cation if they could be shown to have some inhibitory action on the phenomenon. The results obtained permitted to show that in fact, guanidines inhibit K^+ transport, and besides, that this inhibition is of the competitive type, in agreement with the similarity of guanidines to K^+. It could be shown also that guanidines inhibit K^+ transport without altering H^+ extrusion, which agrees with the postulation of two linked but independent systems for the uptake of K^+ and the extrusion of H^+ in yeast.

2. Experimental

Cells were grown and prepared according to the general procedures described before [7], but growth took place under vigorous aeration, in order to obtain cells with a high aerobic metabolism, capable of supporting K^+ uptake with ethanol as substrate. K^+ uptake was measured with a Beckman cationic electrode, oxygen was monitored with a Clark electrode from Yellow Springs Instrument Co., and Rb^+ uptake was measured with ^{86}Rb, as described in the legend for fig. 3.

3. Results and discussion

Fig. 1 shows that, as expected, K^+ uptake by yeast is inhibited by low concentrations of both octyl guanidine and decamethylene biguanidine (synthalin). However, in the simultaneous recording of K^+ and pH it was found that, although synthalin, with ethanol as substrate could inhibit both K^+ transport and H^+ extrusion, with glucose as substrate, only K^+ uptake was inhibited. With octyl guanidine, on the other hand, with both substartes, only K^+ uptake was inhibited.

The effects of both guanidines on these parameters showed a difference on aerobic conditions with ethanol as substrate from which it could be expected that synthalin behaved either as an inhibitor of respiration, or as an uncoupler of oxidative phosphorylation. The results obtained (fig. 2) show that with glucose as substrate both substances inhibit respiration after an initial stimulation. With ethanol as substrate, on the other hand, octyl guanidine caused an initial stimulation of about 30%, and after some time, an ihibition of respiration took place. Synthalin stimulated respiration over the whole length of the experimental period. From these data it seems clear that both guanidines can inhibit respiration, but this inhibition is a late effect that can be better appreciated with glucose as

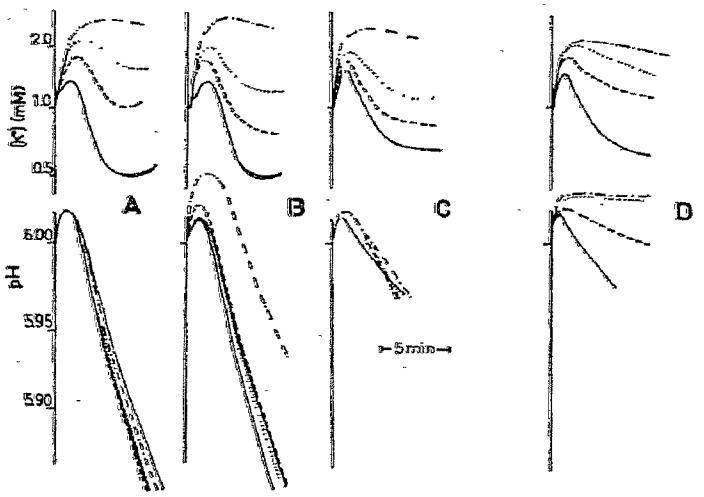

Fig. 1. Effect of octylguanidine and synthalin on K^+ uptake and H^+ extrusion in yeast with ethanol or glucose as substrates. Incubation conditions: 100 mM glucose, or 86 mM ethanol; 40 mM maleic-triethanolamine buffer, pH 6.0; 1 mM KCl; yeast cells, 1.0 g, wet wt; Final volume, 10.0 ml. Temperature, 30°C. The tracings were initiated by the addition of the yeast suspension. A) Synthalin, glucose; B) octylguanidine, glucose; C) octylguanidine, ethanol; D) synthalin, ethanol. The concentrations of synthalin were 0 (——), 0.104 mM (– – – –), 0.279 mM (·····) or 0.695 mM (–·–·–·–). The concentrations of octylguanidine were ○ (——), 0.136 mM (– – – –), 0.364 mM (·····) and 0.908 mM (–·–·–·–).

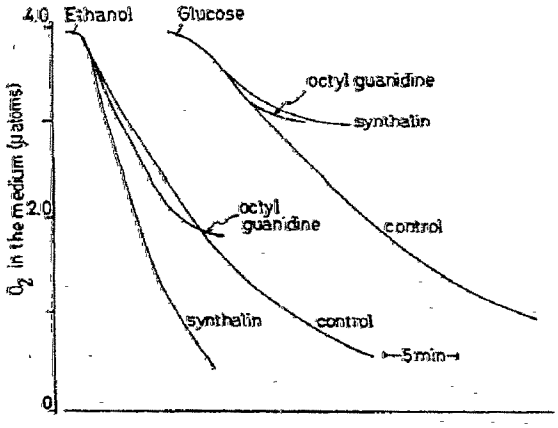

Fig. 2. Effect of 0.354 mM synthalin or 0.456 mM octyl guanidine on respiration with glucose or ethanol as substrates in yeast. Incubation conditions were as for fig. 1, expect that 25 mg of yeast, wet wt., were used.

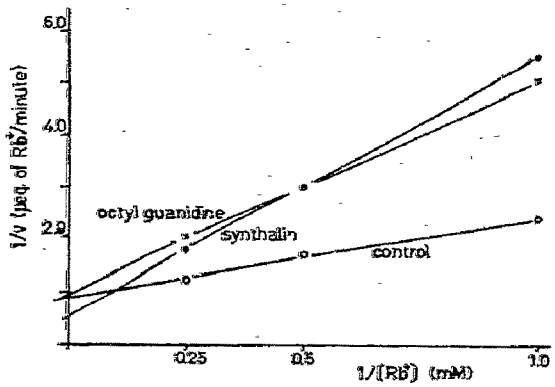

Fig. 3. Double reciprocal plots to show the effects of 0.354 mM synthalin or 0.456 mM octyl guanidine on $^{86}Rb^+$ transport in yeast. The incubation mixture was the same as for fig. 1, but 100 mg of yeast were used. Exactly 1 min after the addition of the yeast suspension, $^{86}RbCl$ was added at the indicated concentrations; after 1 min more, exactly, an aliquot of the mixture was filtered through a 0.45 micron Millipore filter and washed several times. The cells on the filter were resuspended in water and an aliquot was plated and counted.

substrate, and in any case, glycolysis is able to support the energy requirements of the cell in the absence of respiration, as shown by the persistence of the H^+ extrusion in the presence of both inhibitors when glucose was the substrate. With ethanol as substrate, on the other hand, octyl guanidine has a late inhibitory effect on respiration which apparently does not show in short time experiments, and synthalin has an uncoupling effect which, with ethanol as substrate, possibly depletes the energy stores of the cell. The experiments seems to agree with the idea that guanidines can inhibit

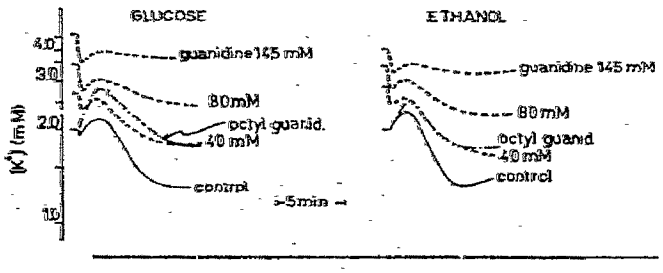

Fig. 4. Comparative effects of octyl guanidine 0.456 mM (–·–·–·–) and guanidine-HCl (– – – –) at several concentrations on K^+ transport in yeast. Incubation conditions were the same as for fig. 1, but 80 mM maleate-triethanolamine, pH 6.0, and 2.0 mM KCl were used. Final volume was 5.0 ml. Control tracing: (——).

K^+ transport without inhibiting H^+ extrusion, provided an adequate energy source is functioning in the cell, and these systems seem to be able to function independently from one another.

Another important question to solve was the nature of the inhibition of K^+ transport produced by the guanidines, in view of the resemblances reported between guanidines and monovalent ions [5]. For the sake of convenience, ^{86}Rb uptake was measured both in the absence and presence of guanidines. Experiments were carried out to measure the K_m of transport and the type of inhibition produced by both guanidines. As expected from the relative resemblances reported [5], the inhibition observed was of the competitive type (fig. 3) with Rb^+; with synthalin, besides, an increase of V_m took place in this experiment, which, however, did not show consistently in other experiments, and seemed to depend on the K^+ content of yeast. A K_i of approx. 0.3 mM for octyl guanidine, and around 0.1 mM for synthalin was calculated from the data in fig. 3.

Although experiments have not been carried out to measure specifically the transport of the guanidines in our experimental system, it is reasonable to assume that they are transported to the interior of the cell from the inhibition observed in respiration, particularly when glucose was used as the substrate. It seems reasonable, besides, to suppose that if both guanidines and Rb^+ compete for the same site, they are transported by the same carrier into the cell.

In relation to the chemical characteristics of both compounds, it is important, first, that both inhibitors are substituted with a long carbon chain. As found by Pressman [2] for the inhibition of mitochondrial metabolism, in our system the same inhibition of K^+ transport could be demonstrated with unsubstituted guanidine, but much higher concentrations were required (fig. 4). The effects on K^+ transport are membrane phenomena, and so it seems that besides the interaction of substituted guanidines with the monovalent cation carrier, the hydrophobic tail is necessary to magnify the inhibition which can be observed with the unsubstituted guanidine. In the figure it can be appreciated that there exists a great qualitative difference in the effects of both guanidine and octyl guanidine; the latter is about 100 times more potent than guanidine. As pointed out by Schäfer and Bojanowski [4] for the action of biguanides, the ability of the molecules to bind to the membrane, which depends on the hydrophobic moiety of the molecule, is an important fact in the magnitude of the effect.

In summary, substituted guanidines are molecules which inhibit K^+ transport probably by interacting with its carrier with the following characteristics: i) the molecules seem to be kinetically similar to K^+; ii) the hydrophobic component of the molecule plays an important role in the enhancement of the effect, in comparison to guanidine; iii) the molecules not only interact with the transport system, but are also transported into the cell, probably by the same carrier as K^+, and iv) the effect of guanidines on K^+ transport can be separated from its effect on H^+ extrusion, which supports our previous idea that H^+ extrusion and K^+ uptake are carried out by two different systems in the yeast cell which are coupled, but can be separated under certain conditions.

References

[1] Martensson, J. (1946) Acta Med. Scand. 32, 125.
[2] Pressman, B.C. (1963) J. Biol. Chem. 238, 401.
[3] Chapell, J.B. (1963) J. Biol. Chem. 238, 410.
[4] Schafer, G. and Bojanowski, D. (1972) European J. Biochem. 27, 364.
[5] Hille, B. (1971) J. Gen. Physiol. 58, 599.
[6] Peña, A., Cinco, G., Gómez-Puyou, A. and Tuena, M. (1972) Arch. Biochem. Biophys. 153, 413.
[7] Peña, A., Cinco, G., Gómez-Puyou, A. and Tuena, M. (1969) Biochim. Biophys. Acta 180, 1.