The Effect of Potassium Ionophores and Potassium on Cellular Calcium in the Yeast *Saccharomyces cerevisiae*

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The efflux of Ca^{2+} from the yeast *Saccharomyces cerevisiae* is induced by the influx of mono- or divalent cations. The mechanism of the tight coupling between K^{+} influx and Ca^{2+} efflux was investigated. Two possibilities were examined: (1) Ca^{2+} efflux is induced only by the carrier-mediated and energy-dependent K^{+} uptake or (2) Ca^{2+} efflux can be induced by electric coupling to K^{+} influx which is driven by its inward electrochemical gradient in the presence of K^{+} ionophores. It was found that K^{+} influx in the presence of valinomycin or nigericin and in the absence of glucose did not induce Ca^{2+} efflux. It is suggested, therefore, that the coupling is mediated by antiport carriers which exchange Ca^{2+} and K^{+} .

INTRODUCTION

Recent studies in our laboratory on the yeast *Saccharomyces cerevisiae* have shown that only 20% of cellular Ca^{2+} was extruded from the cells during incubation for 24 h in medium containing a metabolic substrate but no permeable cations. The presence of K^{+} or Na^{+} in the medium induced a substantial, energy-dependent efflux of Ca^{2+} from the cells (Eilam, 1982*a*). Similar results have been obtained in the presence of magnesium or calcium in the medium (Eilam, 1982*b*). The extrusion of Ca^{2+} , induced by mono- or divalent cations, was accompanied by the uptake of these cations into the cells.

In the present study, the mechanism of K^{+} -induced Ca^{2+} efflux was investigated. The tight coupling between K^{+} uptake and Ca^{2+} efflux may have two explanations: (1) both fluxes may be mediated by the same non-electrogenetic carrier requiring loading of cations on either side before transfer can occur ($\text{K}^{+}/\text{Ca}^{2+}$ antiporter), or (2) the efflux of Ca^{2+} may be electrically coupled to the influx of K^{+} , but mediated by different carriers.

An attempt has been made to distinguish between these two possible mechanisms by the following experiments. In yeast, K^{+} uptake is a carrier-mediated, energy-requiring process (Conway & Duggan, 1958; Armstrong & Rothstein, 1967; Peña, 1975; Derks & Borst-Pauwels, 1980). However, K^{+} influx can also be induced by the addition of a K^{+} ionophore and maintaining a high concentration of K^{+} in the medium. The inward concentration gradient of K^{+} would drive, in the presence of the ionophore, a flux of K^{+} into the cells. If Ca^{2+} efflux is coupled to K^{+} influx electrically (possibility 2), such flux will cause efflux of Ca^{2+} from the cells. If the coupling is non-electrogenic (possibility 1), such flux will diminish Ca^{2+} efflux, since it would diminish the carrier-mediated K^{+} influx.

The results of the present work led to the conclusion that calcium efflux is induced only by the energy-dependent carrier-mediated influx of K^{+} .

METHODS

Organism and culture conditions. *Saccharomyces cerevisiae* strain 123 (genotype MAT a, *his-1*) was maintained at 4 °C on a medium containing (g l^{-1}): agar, 15; yeast extract, 10; glucose, 20 and peptone, 20. Prior to the experiments, cells were inoculated into 40 ml medium that contained (g l^{-1}): Bacto yeast extract, 10; Bacto peptone 20 and glucose, 20. For $^{45}\text{Ca}^{2+}$ efflux experiments $^{45}\text{CaCl}_2$ ($1 \mu\text{Ci ml}^{-1}$; 37 kBq ml^{-1}) was added to the medium. The yeasts were grown overnight in 250 ml flasks with shaking at 200 r.p.m. at 30 °C.

Efflux studies. Cells grown with $^{45}\text{Ca}^{2+}$ were collected by centrifugation and washed by resuspension and recentrifugation in distilled water. The efflux was initiated by suspending the cells at a final concentration of 5×10^7 cells ml^{-1} , in media containing 20 mM-HEPES buffer, pH 5.2 and the indicated cations, substrates and ionophores. The media were equilibrated for 15 min at 30°C with continuous shaking before the addition of the yeast cells, and shaken at 30°C throughout the experiment. Samples (1 ml) were taken immediately after the addition of the yeast and at the indicated times until the end of the experiment. The samples were rapidly filtered on Sartorius membrane filters (0.45 μm pore size), which had been prewashed with 20 mM- MgCl_2 . The cells on the filter were quickly washed three times with 20 mM- MgCl_2 (20 ml). It was previously reported (Boutry *et al.*, 1977) and later confirmed in the yeast *S. cerevisiae* (Y. Eilam, unpublished results) that under these conditions, the amount of $^{45}\text{Ca}^{2+}$ absorbed to the cells when incubated at 2°C is very small. The filters were dried at 65°C and the radioactivity determined in a scintillation fluid containing 3 g PPO and 0.1 g POPOP in 1 litre of toluene. The results are expressed as a percentage of the initial radioactivity present in the cells.

Determination of the cellular content of cations. Cells were collected from an overnight culture by centrifugation, washed by resuspension in distilled water, and resuspended in the indicated medium at a final concentration of 5×10^7 cells ml^{-1} . The suspensions were incubated at 30°C with continuous shaking for the indicated times. After incubation, 2 ml samples of cell suspensions were filtered on to Sartorius membrane filters, prewashed with distilled water. The cells were washed once with 0.5 M-KCl and four times with 5 ml distilled water in less than 2 min. Blank filters which were washed similarly to the samples yielded less than 10% of the amount of K^+ in the samples. These values were subtracted from the sample values.

After filtration, each filter was immersed in 3 ml distilled water, boiled to release the ions from the cells and centrifuged to precipitate the debris. K^+ and Ca^{2+} were determined using a Perkin-Elmer atomic absorption spectrometer after appropriate dilution.

$^{45}\text{CaCl}_2$ [20 mCi (mg Ca^{2+}) $^{-1}$] was purchased from Amersham, valinomycin from Sigma and nigericin from Calbiochem.

RESULTS

The effect of the K^+ ionophore on cellular calcium was examined in the absence of metabolic substrates and in the presence or absence of 0.2 M-KCl in the medium. In the absence of KCl, about 20% of cellular calcium left the cells in 5 h. A slight inhibition of Ca^{2+} efflux was observed in the presence of valinomycin (Fig. 1*a*). KCl (0.2 M) caused a slight stimulation of Ca^{2+} efflux which was prevented by valinomycin (Fig. 1*b*). When glucose was added to the medium (Fig. 2*a, b*), the efflux of calcium was greatly stimulated by the presence of 0.2 M-KCl (Fig. 2*b*), as reported previously (Eilam, 1982*a*). A small but significant inhibition of this efflux was observed in the presence of valinomycin (Fig. 2*a, b*).

It is clear from Figs 1 and 2 that incubating cells in medium containing K^+ ionophore and 0.2 M-KCl did not induce additional efflux of calcium from the cells. In further experiments, the concentration of KCl was increased to 0.5 M, in order to ascertain the inward concentration gradient of potassium. Antimycin A was included in the medium to block the utilization of endogenous substrates.

The results in Table 1 indicate that, even at this high concentration, the presence of valinomycin did not cause Ca^{2+} efflux from the cells. Nigericin is an ionophore that generates an electroneutral exchange of K^+ and H^+ across cell membranes (Harold *et al.*, 1974). Table 1 shows that in the presence of nigericin and 0.5 M-KCl no Ca^{2+} efflux was observed. Deter-

Table 1. *Effects of K^+ ionophores on cellular calcium and potassium levels after 3 h incubation*

Medium*	Cell calcium [nmol (mg dry wt) $^{-1}$]	Cell potassium [nmol (mg dry wt) $^{-1}$]
No addition	2.5 $\pm$ 0.10	395 $\pm$ 13
+ KCl	2.4 $\pm$ 0.09	398 $\pm$ 17
+ KCl + valinomycin (18 μM)	2.8 $\pm$ 0.11	642 $\pm$ 22
+ KCl + nigericin (17 μM)	2.7 $\pm$ 0.20	1185 $\pm$ 31
+ KCl + glucose (0.1 M)	0.8 $\pm$ 0.04	684 $\pm$ 21

* The medium contained HEPES buffer (20 mM) pH 5.2 and Antimycin A (15 μM). The concentration of KCl, when added, was 0.5 M.

Fig. 1. Effect of valinomycin on cell calcium in the absence of glucose. Calcium loss was determined in the absence (a) or presence (b) of 0.2 M-KCl, both without (●) or with (○) 18 μM-valinomycin. The medium contained HEPES (20 mM) pH 5.2. The values are means of four determinations $\pm$ S.E.M.

Fig. 2. Effect of valinomycin on cell calcium in the presence of glucose. Calcium loss was determined in the absence (a) or presence (b) of 0.2 M-KCl, both without (●) or with (○) 18 μM-valinomycin. The medium contained HEPES (20 mM) pH 5.2 and glucose (100 mM). The values are means of four determinations $\pm$ S.E.M.

mination of K^+ content of the cells indicated that both valinomycin and nigericin induced a marked influx of K^+ into the cells (Table 1).

Under similar conditions, the addition of glucose to the medium, in the absence of K^+ ionophores, induced an energy-dependent influx of K^+ into the cells, which was accompanied by an efflux of Ca^{2+} (Table 1).

DISCUSSION

The results indicate that Ca^{2+} efflux is induced by K^+ influx only when the latter is taken up by an energy-dependent, carrier-mediated process. When K^+ influx was driven by its inward electrochemical gradient, in the absence of glucose and in the presence of valinomycin, Ca^{2+} efflux was not induced. In the presence of glucose in the medium, this ionophore inhibited the substantial potassium-dependent Ca^{2+} efflux.

In metabolizing yeasts, K^+ influx is coupled to H^+ extrusion (Ryan & Ryan, 1972; Peña, 1975). In non-metabolizing yeasts incubated in a medium that contains a high concentration of K^+ and nigericin (which generates K^+/H^+ exchange), K^+ influx is also coupled to H^+ efflux.

Therefore, the values of the membrane potential ($\Delta\psi$) and the pH gradient (ΔpH) across the membrane in the presence of nigericin would be similar to those in metabolizing cells. Yet, in the presence of nigericin no Ca^{2+} efflux was observed. These results lead to the suggestion that the energy-dependent K^{+} -induced Ca^{2+} efflux is mediated by carriers which exchange Ca^{2+} for K^{+} , i.e. $\text{K}^{+}/\text{Ca}^{2+}$ antiporters (possibility 1 in the Introduction). The results do not support the second possibility, i.e. that Ca^{2+} efflux is driven by electric coupling to the influx of K^{+} .

Since Na^{+} and K^{+} share the same carrier system in yeast (Armstrong & Rothstein, 1967; Conway & Duggan, 1958; Rothstein, 1974) and since Ca^{2+} efflux is stimulated by Na^{+} uptake in a similar way as by K^{+} uptake (Eilam, 1982a), it is reasonable to suggest that the postulated antiporter can exchange intracellular Ca^{2+} for both K^{+} and Na^{+} .

It should be noted that whereas about 80% of cellular Ca^{2+} leaves the cells via the $\text{Ca}^{2+}/\text{K}^{+}$ antiporter, only a very small fraction of the substantial K^{+} influx enters the cells via this pathway. This conclusion is indicated by the molar ratio of 170 between K^{+} influx and Ca^{2+} efflux in the presence of glucose (Table 1).

The influx of Mg^{2+} , Mn^{2+} and Co^{2+} into yeast cells is accompanied by the efflux of 2 K^{+} for each divalent cation taken up (Lichko *et al.*, 1980; Norris & Kelly, 1977). It has also been suggested that Ca^{2+} uptake is accompanied by K^{+} efflux (Fuhrmann & Rothstein, 1968). However, the mechanisms of these exchange systems were not determined.

An interesting comparison with Ca^{2+} transport across the inner membranes of mammalian heart mitochondria may be pointed out: here, Ca^{2+} efflux is induced by Na^{+} in the medium, and antiporters which exchange Ca^{2+} and Na^{+} have been demonstrated (Crompton & Heid, 1978).

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