Changes in ^{45}Ca and ^{109}Cd Uptake, Membrane Potential and Cell pH in *Saccharomyces cerevisiae* Provoked by Cd^{2+}

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The effect of Cd^{2+} poisoning of *Saccharomyces cerevisiae* on ^{45}Ca , ^{109}Cd and [^{14}C]tetraphenylphosphonium (TPP) uptake and cell pH was examined. At Cd^{2+} concentrations that produced substantial K^+ efflux the rates of uptake of ^{45}Ca , ^{109}Cd and [^{14}C]TPP increased progressively during incubation of the cells with Cd^{2+} , and the cell pH was lowered concomitantly. The initial rates of uptake of the divalent cations and of TPP were increased in cells pre-loaded with Cd^{2+} , which shows that stimulation of the ion fluxes was exerted by the Cd^{2+} that accumulated in the cells. The distribution ratio of TPP between cells and medium, however, was decreased by Cd^{2+} . Although hyperpolarization of the cell membrane by Cd^{2+} cannot be excluded, it is argued that Cd^{2+} primarily stimulated divalent cation uptake by increasing the cation permeability of the cell membrane allowing the cations to enter the cells more easily.

INTRODUCTION

Cd^{2+} is very toxic to all kinds of living cells including yeasts (Norris & Kelly, 1977; Gadd & Mowl, 1983; Kessels *et al.*, 1985; Heldwein *et al.*, 1977; Macara, 1978; Bitton *et al.*, 1984). Not only is growth inhibited but Cd^{2+} also provokes K^+ loss from metabolizing yeast cells (Norris & Kelly, 1977; Gadd & Mowl, 1983; Kessels *et al.*, 1985). Cd^{2+} must first enter the cells before giving rise to a detectable effect (Kessels *et al.*, 1985). Provoking K^+ loss is a property common to a large number of organic poisons, e.g. DIO-9, ethidium, miconazole, trifluoperazine, compound 48/80, calmidazolium and diethylstilboestrol (Foury *et al.*, 1977; Peña & Ramírez, 1976; Peña, 1978; Dufour *et al.*, 1980; Eilam, 1983, 1984; Borst-Pauwels *et al.*, 1983, 1986). These compounds are inhibitors of the yeast plasma-membrane ATPase (Foury *et al.*, 1977; Dufour *et al.*, 1980; Eilam, 1984; Borst-Pauwels *et al.*, 1983, 1986; Serrano, 1980). Furthermore, they all enhance Ca^{2+} or Sr^{2+} uptake. We have now examined whether Cd^{2+} also increases Ca^{2+} uptake.

METHODS

Yeast growth. *Saccharomyces cerevisiae* strain Delft 2 was grown to stationary phase in medium A [1% (w/v) Yeast Extract, 2% (w/v) Bacto-Peptone, 2% (w/v) glucose, 0.2% $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 3.46% (w/v) KH_2PO_4 , 0.04% K_2HPO_4 , brought to pH 4.5 with HCl] as described by Kessels *et al.* (1985).

Uptake of ^{109}Cd and ^{45}Ca . This was studied by adding 37 nM- ^{109}Cd or 1.2 μM - ^{45}Ca to a 2% (w/v) yeast suspension in 45 mM-Tris/succinate buffer, pH 5.0, that had been supplemented with 3% (w/v) glucose 20 min earlier. The specific activities of ^{109}Cd and ^{45}Ca were 0.9 Ci mmol $^{-1}$ (33.3 GBq mmol $^{-1}$) and 9.2 mCi mmol $^{-1}$ (340.4 MBq mmol $^{-1}$), respectively. Nitrogen was bubbled through the suspension at 25 °C and at appropriate times 2 ml samples were filtered and washed (Kessels *et al.*, 1985). Radioactivity on the filters was determined by liquid scintillation counting.

Abbreviation: TPP, tetraphenylphosphonium.

Uptake of TPP (tetraphenylphosphonium). This was determined by the procedure of Boxman *et al.* (1982). After the 2% (w/v) yeast suspension had been pre-incubated for 20 min with 3% (w/v) glucose in 45 mM-Tris/succinate buffer, pH 5.0, as described above, uptake experiments were started by addition of $0.36 \mu\text{M}$ - $[^{14}\text{C}]\text{TPP}$ [specific activity 50 mCi mmol^{-1} ($1.85 \text{ GBq mmol}^{-1}$)].

Cell pH. This was determined by filtering 5 ml samples of 2% (w/v) yeast suspension in Tris/succinate buffer, pH 5.0, washing the cells twice with 2 ml ice-cold water, and freezing the filters in liquid nitrogen (Borst-Pauwels & Dobbelmann, 1972). After thawing, the filters with the cells were boiled for 30 s in 0.5 ml 400 mM-KCl; the pH was then measured with a glass electrode.

Binding of 9-aminoacridine. Binding of this fluorescent dye ($1 \mu\text{M}$) to 5% (w/v) non-metabolizing yeast cells was measured as described by Theuvsen *et al.* (1984) in 45 mM-Tris/succinate buffer, pH 5.0, at 25°C in the absence of glucose.

Chemicals. 9-Aminoacridine was from Sigma; Bacto-Peptone and Yeast Extract were from Difco; radiochemicals were from Amersham; all other chemicals used were reagent grade and were from commercial sources.

RESULTS

At concentrations of 0.005 – 1 mM , Cd^{2+} causes K^+ loss from metabolizing yeast cells (Kessels *et al.*, 1985); we investigated whether this K^+ loss was accompanied by an enhancement of Ca^{2+} uptake. Cd^{2+} (1 mM) gave rise to a much higher uptake of carrier-free ^{45}Ca than was found in the presence of 1 mM non-radioactive Ca^{2+} (Fig. 1). The net rate of ^{45}Ca uptake increased gradually during accumulation of Cd^{2+} reaching a maximum after about 3 min, whereafter it gradually decreased. In the presence of 1 mM non-radioactive Ca^{2+} no increase in the net rate of uptake of ^{45}Ca was observed. Also, the net uptake rate of ^{109}Cd increased gradually during incubation of the cells in the presence of 1 mM non-radioactive Cd^{2+} .

Fig. 1 also shows that the increase in the net uptake rate of ^{45}Ca or ^{109}Cd was due to an increase in the influx rate of the two isotopes rather than to a decrease in the efflux rate. The rates of influx were greatly increased on pre-loading the cells for 5 min with 1 mM - Cd^{2+} before addition of the two isotopes. The net rates of uptake were appreciably lower than the corresponding influx rates. Therefore, there was still appreciable efflux of the two isotopes. The influx rate of ^{45}Ca was not increased on pre-incubating the cells for 5 min with 1 mM - Ca^{2+} .

The dependence of the influx rates of ^{45}Ca or ^{109}Cd on the cellular Cd content showed saturation kinetics (Fig. 2). The maximum increase in the uptake rate was approximately 100%. The cellular Cd content at which half-maximum stimulation occurred was $2.3 \text{ mmol (kg dry wt)}^{-1}$.

The compound trifluoperazine not only stimulates K^+ efflux and Ca^{2+} uptake in metabolizing yeast cells but also increases the equilibrium distribution of the lipophilic cation TPP (Eilam, 1983, 1984). This indicates that yeast cells are hyperpolarized by trifluoperazine, which might be the cause of the increased divalent cation uptake provoked by this compound. Similarly, the enhancements of Sr^{2+} and K^+ fluxes by diethylstilboestrol are also accompanied by an increased equilibrium distribution of TPP (Borst-Pauwels *et al.*, 1984). We therefore examined whether Cd^{2+} also enhances TPP uptake. Fig. 3 shows that 1 mM - Cd^{2+} led to a large efflux of TPP from yeast cells which had been pre-loaded to equilibrium with TPP; 1 mM - Ca^{2+} also gave rise to an efflux of TPP. This efflux, however, was much smaller than the efflux provoked by Cd^{2+} .

We considered the possibility that the efflux of TPP was due to a displacement by Cd^{2+} of TPP bound to intracellular binding sites, rather than to a depolarization of the cells. Theoretically, the initial rate of entry of TPP into the yeast cells should not depend on the extent to which TPP is bound inside the cell. Therefore, we also determined the effect of Cd^{2+} on the initial rate of influx of TPP into the cells. The influx rate of TPP uptake into the cells was higher in the presence of 1 mM - Cd^{2+} than in the presence of 1 mM - Ca^{2+} (Fig. 4). Typically, the uptake rate of TPP increased gradually during incubation of the cells with Cd^{2+} . Accordingly, the influx rate of TPP found when TPP was added 5 min after the addition of Cd^{2+} was much greater than the rate found on adding TPP and Cd^{2+} together.

Theoretically, the influx rate depends not only on the membrane potential but also on the surface potential. A reduction in the negative surface potential of the yeast caused by the

Fig. 1. Comparison of ^{45}Ca and ^{109}Cd uptake by metabolizing cells in the presence of 1 mM- Cd^{2+} or 1 mM- Ca^{2+} and effect of pre-loading the cells with Ca^{2+} or Cd^{2+} . $\square$, ^{45}Ca uptake in the presence of 1 mM- Cd^{2+} ; $\bullet$, ^{109}Cd uptake in the presence of 1 mM- Cd^{2+} ; $\circ$, ^{45}Ca uptake in the presence of 1 mM- Ca^{2+} . The carrier-free isotopes were added either together with the non-radioactive divalent cations or 5 min after the addition of these cations.

Fig. 2

Fig. 3

Fig. 2. Dependence of the influx rate of ^{45}Ca and ^{109}Cd on the intracellular Cd content. The influx rates were obtained by adding the radioactive isotopes after various periods of incubation of the cells with 1 mM- Cd^{2+} . $\circ$, ^{45}Ca uptake; $\bullet$, ^{109}Cd uptake.

Fig. 3. Effect of 1 mM- Cd^{2+} or 1 mM- Ca^{2+} on the cellular TPP content of metabolizing cells that had accumulated TPP for 3 h. $\bullet$, Cd^{2+} added; $\circ$, Ca^{2+} added; $\square$, control (no additions).

addition of divalent cations will lead to a decrease in the influx rate of TPP, because the interfacial concentration of TPP near the plasma-membrane is reduced. Binding of the monovalent cationic dye 9-aminoacridine to the yeast cells is proportional to the interfacial monovalent cation concentration near the plasma-membrane (Theuvsen *et al.*, 1984). The binding of 9-aminoacridine to the yeast cells was reduced more by 1 mM- Cd^{2+} than by 1 mM- Ca^{2+} , namely to $43 \pm 5\%$ and $61 \pm 5\%$ of the control value, respectively. (Experiments were done in triplicate; values are means $\pm$ SEM.) From the 9-aminoacridine binding studies it can be concluded that 1 mM- Cd^{2+} also decreased the interfacial concentration of TPP more than did

Fig. 4

Fig. 4. Uptake of TPP by metabolizing yeast cells – effect of 1 mM- Cd^{2+} or 1 mM- Ca^{2+} . TPP was added either together with divalent cations or after 5 min incubation of the cells with the divalent cations. ●, 1 mM- Cd^{2+} added; ○, 1 mM- Ca^{2+} added; □, no divalent cation added. Inset: TPP uptake after 5 min incubation with 1 mM- Cd^{2+} .

Fig. 5

Fig. 5. Effect of 1 mM- Cd^{2+} (●) or 1 mM- Ca^{2+} (○) on the cell pH of metabolizing yeast.

1 mM- Ca^{2+} , and that the initial rate of TPP influx should be corrected for this differential decrease in interfacial TPP concentration. The corrected influx rates of TPP appeared to be $338 \pm 5\%$ (Cd^{2+}) and $161 \pm 31\%$ (Ca^{2+}) of the influx rate found in the absence of added divalent cation. This shows that pre-incubation of the cells with Cd^{2+} increased the initial rate of TPP influx more than did pre-incubation with Ca^{2+} . When TPP was added together with the divalent cations, Ca^{2+} did not significantly affect the TPP influx rate, whereas Cd^{2+} increased that rate twofold.

Ethidium, which also causes simultaneous enhancement of K^+ loss and Ca^{2+} uptake (Peña, 1978) leads to an acidification of the cells (Peña & Ramírez, 1976), and 1 mM- Cd^{2+} had a similar effect (Fig. 5). On the other hand, 1 mM- Ca^{2+} did not really lead to a change in cell pH.

DISCUSSION

The property, shared by a large number of organic poisons such as ethidium, trifluoperazine, compound 48/80, calmidazolium, miconazole and diethylstilboestrol (Eilam, 1983; Borst-Pauwels *et al.*, 1984, 1986; Serrano, 1980), of provoking K^+ efflux and concomitantly enhancing Ca^{2+} uptake in metabolizing yeast cells, is also exhibited by Cd^{2+} . This may indicate that Cd^{2+} interferes with yeast cells in a similar way to these organic compounds. All these compounds inhibit the yeast plasma-membrane ATPase (Dufour *et al.*, 1980; Eilam, 1984; Borst-Pauwels *et al.*, 1986; Serrano, 1980), as does Cd^{2+} (Ahlers & Rösick, 1985).

Whether the hyperpolarization of the plasma-membrane is a common effect of the organic poisons is less certain. Until now, only trifluoperazine and diethylstilboestrol have been proven to cause hyperpolarization (Eilam, 1984; Borst-Pauwels *et al.*, 1984; Eilam *et al.*, 1985). The other organic poisons, DIO-9, ethidium, miconazole, compound 48/80 and calmidazolium, do not increase the intracellular concentration of TPP at equilibrium; they only increase the influx rate of TPP (G. W. F. H. Borst-Pauwels, unpublished). The efflux of TPP from cells pre-loaded with TPP, as provoked by Cd^{2+} , may be due to a displacement by Cd^{2+} of TPP bound to

intracellular binding sites, rather than to depolarization of the cells. Some support for this view is provided by the fact that the initial rate of influx of TPP into the cells in the presence of 1 mM- Cd^{2+} is higher than that in the presence of 1 mM- Ca^{2+} .

A hyperpolarization of the plasma-membrane may account for the increase in the rate of ^{45}Ca uptake caused by Cd^{2+} . On the other hand, the increase in TPP uptake observed after 5 min pre-incubation of the cells with 1 mM- Ca^{2+} is not accompanied by an increased rate of ^{45}Ca influx. This shows that an increase in TPP influx rate is not necessarily accompanied by an increase in the rate of divalent cation uptake. Furthermore, as will be shown elsewhere, the increase in Ca^{2+} uptake provoked by trifluoperazine, a compound which hyperpolarizes the yeast cells (Eilam, 1984), is not due to this hyperpolarization but to an increase in the cation permeability of the cells. Therefore, an alternative explanation for the increased rate of ^{45}Ca influx provoked by Cd^{2+} is that the permeability of the cells is increased by Cd^{2+} . That permeabilizing the cells can lead to increased cation uptake is shown by the fact that DEAE-dextran, which permeabilizes the plasmalemma specifically without affecting the tonoplast, also increases the influx rate of Ca^{2+} (Theuvenet *et al.*, 1986). A third possible explanation for the enhancement of ^{45}Ca uptake by 1 mM- Cd^{2+} is the acidification of the cells caused by Cd^{2+} . A decrease in cell pH does indeed lead to an increase in divalent cation uptake (Roomans *et al.*, 1979).

As we have shown (Kessels *et al.*, 1985) the dependence of the K^+ efflux provoked by Cd^{2+} on the intracellular Cd^{2+} content exhibits saturation kinetics. We have now shown that the dependence of the rate of ^{109}Cd or ^{45}Ca influx on the intracellular Cd content also shows saturation kinetics. This supports the notion that saturable sites are involved in the interaction of Cd^{2+} with the yeast cell. Neither the precise location inside the cells nor the nature of the binding sites are known at this stage. The similarity in the dependence of K^+ efflux on the cellular Cd content and the dependence of Ca^{2+} influx on cellular Cd supports the view that both processes are closely related, in accordance with earlier findings (Kessels *et al.*, 1985). The half-maximum Cd^{2+} concentrations are approximately the same.

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