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THE EFFECT OF MONOVALENT CATIONS ON CALCIUM EFFLUX IN YEASTS

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The properties of the calcium efflux system in the yeast *Saccharomyces cerevisiae* were investigated. After growing the cells overnight in medium containing ^{45}Ca, the cells were transferred to medium containing glucose, Hepes buffer (pH 5.2) and monovalent cations. The presence of potassium or sodium in the medium induced efflux of calcium from the cells. The magnitude of the efflux was dependent on the concentration of these cations in the medium. The time course of calcium efflux was analyzed, and two types of exchangeable calcium pools, which turned over at different rates, were detected: 'Fast turnover' and 'slow turnover'. Increase in the concentration of monovalent cations in the medium caused an increase in the fraction of cellular calcium which turned over at a fast rate, and activation of calcium efflux from the 'slow turnover' calcium pool. The specific changes in the parameters of calcium efflux induced by monovalent cations were different from those reported previously to be induced by divalent cations. Both processes, i.e. activation of calcium efflux by monovalent and by divalent cations, were found to be additive, indicating that they operate via different mechanisms. Experiments using the respiratory inhibitor Antimycin A, showed that stimulation of calcium efflux by monovalent cations is energy dependent. Lanthanum ions which are known to inhibit calcium influx into yeast cells, inhibitted the activation of calcium efflux by both divalent and monovalent cations. Determination of the cationic composition of the cells indicated that the stimulation of calcium efflux was accompanied by influx of potassium or sodium into the cells.

Introduction

In a previous work we have investigated the properties of calcium efflux system in the yeast *Saccharomyces cerevisae* [1]. Kinetic analysis of ^{45}Ca efflux curves indicated the presence of two exchangeable intracellular calcium pools which turned over at different rates: a 'fast turnover' and a 'slow turnover'. At an acidic pH (5.2) and in the absence of calcium or magnesium in the medium only 20% of cellular calcium left the 'fast turnover' calcium pool. No efflux from the 'slow-turnover' calcium pool was detected. The presence of magnesium or calcium in the medium caused calcium efflux from the 'slow-turnover' calcium pool. The rates of efflux were dependent on the concentration of these ions in the medium.

In the present work we investigated the effect of monovalent cations on calcium efflux. It is well established that yeast cells take up potassium and sodium with great rapidity. This uptake is mediated by a saturable, energy-dependent, monovalent-cation carrier [2–4] which may also mediate the efflux of potassium and sodium from the cells [5,6]. The efflux of K^+ and Na^+ is accelerated by the presence of monovalent cations in the medium, in which case a stoichiometric cation-exchange occurs [6].

It is clear to date that divalent cations are

Abbreviation Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

transported into yeast cells by a distinct carrier system, different from the monovalent carrier [7]. However, some interactions between monovalent cations and divalent-cation carriers and vice versa have been reported: The presence of potassium in the medium suppressed calcium uptake at all concentrations [8,9], but the presence of sodium in the medium suppressed calcium uptake only at low concentrations and activated it at high concentrations [8]. The presence of calcium in the medium suppressed potassium uptake in a non-competitive manner [2] and Ca^{2+} also inhibited Rb^{+} uptake in an apparently competitive way [27]. The influx of magnesium, manganese, cobalt and cadmium into yeast cells was accompanied by efflux of potassium [10,11]. The mechanisms of these interactions are not yet understood.

In the present work we have found that calcium efflux is activated by the presence of monovalent cations in the medium. This activation is energy dependent and can be inhibited by low concentrations of lanthanum chloride.

Methods

Organism and culture conditions. Saccharomyces cerevisiae strain 123 (genotype MAT a, his 1) (generously provided by Dr. G. Simchen, The Hebrew University), was maintained at 4°C on 1.5% w/v agar containing 1% yeast extract, 2% glucose and 2% peptone. Prior to the experiments, cells were inoculated in a medium containing Bacto yeast extract (10 g/l), Bacto peptone (20 g/l) and glucose (20 g/l) (Medium I). For ^{45}Ca efflux experiments $^{45}CaCl_2$ (1 μCi/ml) was added to medium I. The yeast cells were grown overnight in shaking flasks at 30°C.

Efflux studies. The yeast cells grown with $^{45}Ca^{2+}$ were collected by centrifugation and washed by resuspension in distilled water. The efflux was initiated by suspending the yeast cells at a final concentration of $5 \cdot 10^7$ cell/ml, in media containing 20 mM Hepes buffer (pH 5.2), 100 mM glucose and the indicated cations or inhibitors. 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 of 1 ml of cell suspensions were taken immediately after the addition of the yeasts and at the indicated times until the end of the experiment at 27 h. 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 ml of 20 mM $MgCl_2$. It was previously reported [1,12] that under these conditions the amount of ^{45}Ca adsorbed to the cells when incubated at 2°C is very small. The filters were dried and the radioactivity determined in toluene-containing scintillation fluid. The results are expressed as fraction of the initial radioactivity present in the cells at each time point.

Determination of cation content. Yeast cells were grown overnight in medium I. The cells were then collected, washed twice by resuspension in distilled water and resuspended in 20 ml of medium containing 20 mM Hepes (pH 5.2), 100 mM glucose and the indicated cation. The suspensions were shaken at 30°C for 24 h. The yeast cells were then collected by centrifugation, washed twice with distilled water and the pellets were digested overnight in 3 ml 1:2 mixture of 60% perchloric acid and 30% H_2O_2. The debris was spun down and the supernatants were diluted with a solution containing $LaCl_3$ (0.75 mg/ml final concentration). The dilutions were adjusted so that the concentration of potassium, sodium and calcium was in the range of linear response for each cation. One part of the cells grown overnight in medium I was washed twice with distilled water and immediately digested for the determination of cation content at zero time. Standard solutions of calcium, potassium and sodium used for calibration also contained $LaCl_3$ (0.75 mg/ml) and the same amount of perchloric acid-H_2O_2 mixture as in the cell digest.

The cell number was determined in cell suspensions using a hemocytometer after appropriate dilution.

$^{45}CaCl_2$ (20 mCi/mg calcium) was purchased from Amersham International, U.K.

Results

The yeast cells were equilibrated with ^{45}Ca by growing them for 19 h in medium I containing 1 μCi/ml ^{45}Ca. When the ^{45}Ca-loaded cells were transferred to a medium containing 20 mM Hepes

(pH 5.2) and 100 mM glucose, the cells lost 20% of their cellular calcium during the first two hours. There was no further decrease in the amount of cellular calcium for an additional 25 h (Figs. 1 and 2 and Ref. 1). However, when the medium contained potassium or sodium at different concentrations (2–100 mM), ^{45}Ca left the cells continuously during the 27-h period (Figs. 1 and 2).

The procedure of our experiments involved washing the cells with 20 mM $MgCl_2$ before the determination of the radioactivities. It was shown previously that this procedure removes all the extracellular, cell-wall bound ^{45}Ca [1,12]. Similarly, it was shown in a different study that a wash with 5 mM $CaCl_2$ was sufficient to remove 95% of bound calcium [13]. We assume, therefore, that the radioactivity remaining in the cells represented intracellular calcium. The amount of calcium in the cells was determined from the measured amount of calcium in medium I (4 μg/ml), the radioactivity in the medium in which the cells were grown overnight, and the number of cells in a sample. The amount determined was $(3.75 \pm 0.3) \cdot 10^{-17}$ mol per cell, or 2.6 mmol/kg dry weight.

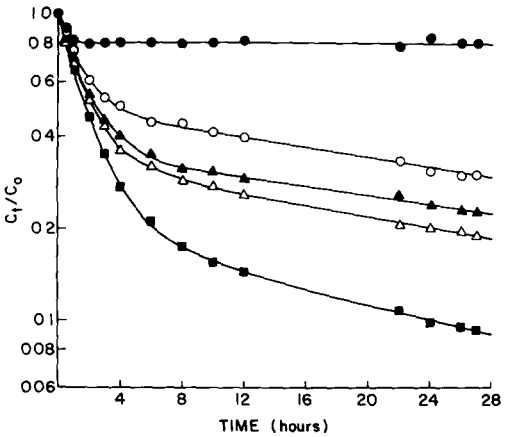


Fig 1 The effect of potassium on ^{45}Ca efflux in yeast cells C_t and C_0 are the radioactivity in the cells at time t and time zero, respectively Medium contained 20 mM Hepes buffer (pH 5 2), 100 mM glucose and the following concentrations of KCl (●) none; (○) 2 mM KCl; (▲) 10 mM KCl, (△) 50 mM KCl; (■) 100 mM KCl The points are the results of a representative experiment and the lines were obtained by computer fitting, using the model of two exponential terms for concentrations between 0–50 mM KCl and three exponential terms for 100 mM KCl Chi square test for the 'goodness of fit' [14] yielded significant fit for all curves ($P<0$ 01)

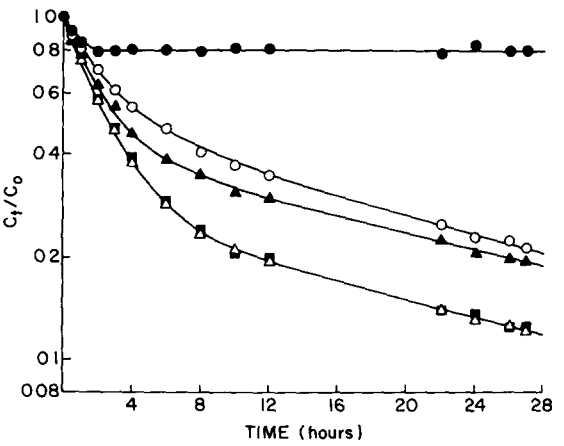


Fig 2 The effect of sodium ions on ^{45}Ca efflux in yeast cells As in Fig. 1, but medium contained NaCl instead of KCl (●) no NaCl, (○) 2 mM NaCl, (▲) 10 mM NaCl, (△) 50 mM NaCl, (■) 100 mM NaCl The points are the results of a representative experiment and the lines were obtained by computer fitting using the model of two exponential terms for concentrations between 0 and 10 mM NaCl and three exponentials for 50 and 100 mM NaCl All curves yielded significant fit

The experiments depicted in Figs. 1 and 2 indicate the presence of more than one compartment for calcium. Therefore, these results were analyzed by a computer program based on a method of non-linear least square fitting and the model of Equation 1.

$$\frac{C_t}{C_0} = \sum_{i=1}^{2\text{or}3} b_i \exp(-\lambda_i t) \qquad (1)$$

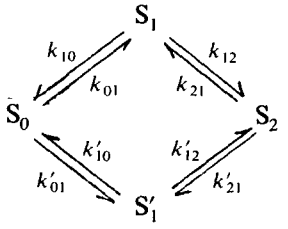
C_t and C_0 are the radioactivities in the cells at time t and time zero, respectively. b_i is the coefficient and λ_i is the exponential constant of the ith exponential term. The results obtained with 2, 10 and 50 mM KCl and with 2 mM and 10 mM NaCl showed a significant fit to a model of two exponential terms ($p<0.01$ for all curves). Chi square values (χ^2) for the best fit were subjected to the F-test for additional terms [14] and yielded no significant improvement of the fit by introducing a third exponential term. The results obtained with 100 mM KCl or 50 mM and 100 mM NaCl yielded significant improvement of the fit ($P<$ 0.05) by introducing a third exponential term. In Figs. 1 and 2, the points represent the experimen-

tal data and the lines were obtained by computer-fitting, yielding the values listed in Table I.

For the calculation of the fluxes and pool sizes, we assumed that the two calcium pools, the 'fast-turnover' (S_1) and the 'slow-turnover' (S_2) were in series [1,15]. It should be noted, however, that since the rates of efflux of the two pools differ by more than one order of magnitude, the model of two compartments in parallel would yield almost identical results. The additional compartment (S'_1) detected in the presence of high concentrations of monovalent cations in the medium turned over at a rate very similar to that of the 'fast-turnover' compartment (S_1). Therefore, we assumed that it represented a subpool in parallel with S_1. The 'parallel' model would be the simplest in the absence of support for a more complicated model. The following schemes were used for calculations:

$$S_0 \underset{k_{01}}{\overset{k_{10}}{\leftrightarrows}} S_1 \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} S_2$$

Two-compartment model



Three-compartment model

S_0 is the medium calcium, S_1 and S'_1 are 'fast-turnover' calcium pools and S_2 is a 'slow-turnover' calcium pool. k_{ij} are the rates constants of fluxes from compartment i to j.

The method for the calculations of the rates, pool sizes and rate constants, according to the solutions of Uchikawa and Borle [15] were described previously [1]. The parameters are listed in Table II.

It is clear from Table II that the increase in the concentration of Na^+ or K^+ in the medium induced the following changes: (1) A change in the distribution of calcium between the pools: an increase in the amount in the 'fast-turnover' pools ($S_1 + S'_1$). (2) Increase in the efflux from the 'fast-turnover' pools ($\rho_{10} + \rho'_{10}$). (3) Decrease in the efflux from S_2 (ρ_{21}), and in the amount in S_2.

In the experiments depicted in Fig. 3 we examined whether changes in calcium fluxes and distribution induced by monovalent cations were additive to the changes induced by divalent cations. It is clear from Fig. 3 that in the presence of 2 mM magnesium and 100 mM KCl or NaCl more calcium left the cells after 27 h than in the presence of magnesium or monovalent cations alone. The parameters obtained by the analysis of the curves (Table II) show that the addition of NaCl or KCl to a medium containing Mg^{2+} caused the following changes: (1) Increase in the size of the fast-turnover calcium pool. (2) The rate of efflux from the fast-turnover calcium pool was higher than the rate in the presence of monovalent cations alone, and slightly higher than in the presence of magnesium alone. (3) The rates of efflux from the slow-turnover calcium pool were lower than those when magnesium alone was present but higher than those when monovalent cations alone were present. These results indicate that the changes induced by monovalent and divalent cations are, to a large extent, additive.

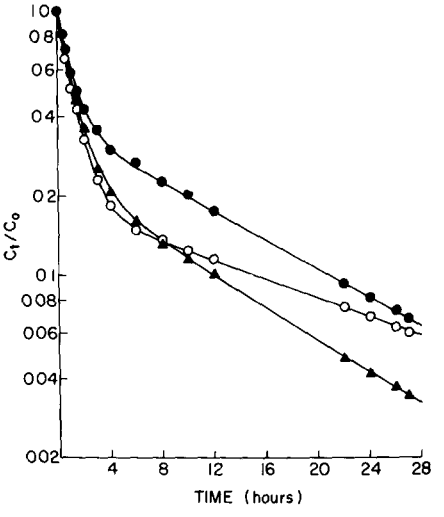


Fig 3 The combined effect of monovalent and divalent cations on ^{45}Ca efflux in yeast cells. The medium contained 20 mM Hepes buffer (pH 5 2), 100 mM glucose, and (●) 2 mM $MgCl_2$ or (○) 100 mM KCl and 2 mM $MgCl_2$ or (▲) 100 mM NaCl and 2 mM $MgCl_2$. The lines were obtained by computer fitting using the model of two exponential terms All curves yielded significant fit ($P<0.01$)

TABLE I

COEFFICIENTS AND EXPONENTIAL CONSTANTS OBTAINED WITH DIFFERENT IONIC COMPOSITIONS OF THE MEDIUM

Medium (Concn in mM)		Slope (h^{-1})			Intercept fraction of cell Ca^{2+}			Intercept (mol/cell) ($\times 10^{17}$) [a]		
		λ_1	λ_1'	λ_2	b_1	b_1'	b_2	b_1	b_1'	b_2
KCl	2	0 68 ± 0 03	–	0 019 ± 0 001	0 49 ± 0 02	–	0 50 ± 0 03	1.83 ± 0 74		1 87 ± 0 03
	10	0 65 ± 0 03	–	0 018 ± 0.001	0 64 ± 0 03	–	0 37 ± 0 01	2 40 ± 0 11		1 39 ± 0 03
	50	0.66 ± 0 04	–	0.020 ± 0 002	0 68 ± 0 03	–	0 33 ± 0 02	2 53 ± 0 11		1 24 ± 0 07
	100	0 82 ± 0 05	0 430 ± 0 04	0 028 ± 0 002	0 31 ± 0 01	0 48 ± 0 02	0 20 ± 0 01	1 16 ± 0 04	1 82 ± 0 07	0 76 ± 0.038
NaCl:	2	0.40 ± 0 02	–	0 032 ± 0 002	0 48 ± 0 03	–	0.51 ± 0 02	1 80 ± 0 11		1.92 ± 0 075
	10	0 49 ± 0.03	–	0 029 ± 0 003	0 60 ± 0 03	–	0 43 ± 0 02	2 24 ± 0 11		1 60 ± 0.04
	50 100	0 50 ± 0.03	0.375 ± 0 02	0 030 ± 0 002	0 34 ± 0 02	0 40 ± 0 01	0 28 ± 0 01	1 26 ± 0 07	1 51 ± 0 04	1 03 ± 0 04
$MgCl_2$.	2	1.02 ± 0.05	–	0.063 ± 0 003	0 62 ± 0 03	–	0 38 ± 0 02	2 34 ± 0 07		1 42 ± 0 03
$MgCl_2$:	2									
+ KCl	100	0 905 ± 0 05	–	0 041 ± 0 003	0 80 ± 0.05	–	0 19 ± 0 01	3 00 ± 0 19		0 71 ± 0.04
$MgCl_2$.	2									
+ NaCl.	100	0 83 ± 0 05	–	0.072 ± 0 004	0 80 ± 0 05	–	0 24 ± 0 02	3 00 ± 0 10		0 91 ± 0 07

[a] To obtain the intercept on a per mg dry weight basis, the values should be multiplied by $7 \cdot 10^7$

TABLE II

CALCULATED RATES, RATE CONSTANTS AND POOL SIZES FOR ^{45}Ca EFFLUX

Medium (Concn in mM)		Rates (mol/cell per h) ($\times 10^{17}$) [a]			Pool size (mol/cell) ($\times 10^{17}$) [a]			Rate constants (h^{-1})				
		ρ_{10}	ρ'_{10}	ρ_{12}	S_1	S'_1	S_2	k_{10}	k'_{10}	k_{12}	k'_{12}	k_{21}
KCl·	2	1 29 ±0 10	–	0.034 ±0.003	1.94 ±0 08		1 76 ±0 04	0 66 ±0 03		0.017 ±0 001		0 019 ±0 001
	10	1 60 ±0.14	–	0.024 ±0.002	2 48 ±0 12		1 31 ±0 04	0 64 ±0 03		0 010 ±0 006		0 018 ±0 001
	50	1 69 ±0 15	–	0 024 ±0 002	2 59 ±0 12		1.18 ±0 08	0.65 ±0 04		0 009 ±0 001		0 020 ±0 002
	100	0 98 ±0.09	0 80 ±0.11	0.020 ±0.002	1 21 ±0 05	1 90 ±0 08	0 70 ±0 04	0 81 ±0.05	0 42 ±0 02	0 016 ±0 001	0 01 ±0 005	0 028 ±0 002
NaCl.	2	0.78 ±0.09	–	0 056 ±0 005	2 10 ±0 14		1 62 ±0 09	0 37 ±0 03		0 027 ±0 002		0 034 ±0.002
	10	1 14 ±0 11	–	0 043 ±0 004	2 41 ±0.14		1 43 ±0 05	0 47 ±0 04		0 018 ±0 001		0 030 ±0 003
	50	0.66 ±0 06	0 60 ±0.05	0 028 ±0.003	1 37 ±0.08	1.68 ±0 05	0 89 ±0 05	0 48 ±0 03	0 35 ±0 01	0 020 ±0 001	0 017 ±0 006	0 031 ±0 002
	100											
$MgCl_2$·	2	2.47 ±0 08	–	0 082 ±0 006	2.48 ±0 08		1 26 ±0 04	0 98 ±0 06		0 033 ±0.003		0 065 ±0 003
$MgCl_2$. +KCl:	2 100	2 74 ±0.20	–	0 027 ±0.002	3 05 ±0.21		0 66 ±0.05	0 90 ±0.05		0 009 ±0 001		0 041 ±0 003
$MgCl_2$: +NaCl·	2 100	2.55 ±0.09	–	0 057 ±0 005	3 14 ±0 21		0 77 ±0.08	0 81 ±0 05		0 008 ±0 001		0 074 ±0 004

[a] To obtain the pool sizes and the rates on a per mg dry weight basis, the values should be multiplied by $7 \cdot 10^7$

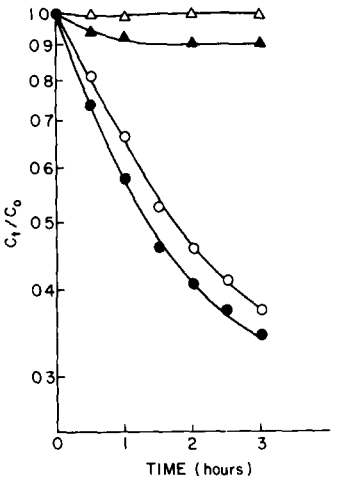


Fig 4 The effect of glucose and of the respiratory inhibitor Antimycin A on ^{45}Ca efflux in yeast cells Medium contained 20 mM Hepes buffer (pH 5 2), 100 mM KCl, and the following: (▲) none, (●) 100 mM glucose; (△) 15 μM Antimycin A, (○) 100 mM glucose + 15 μM Antimycin A

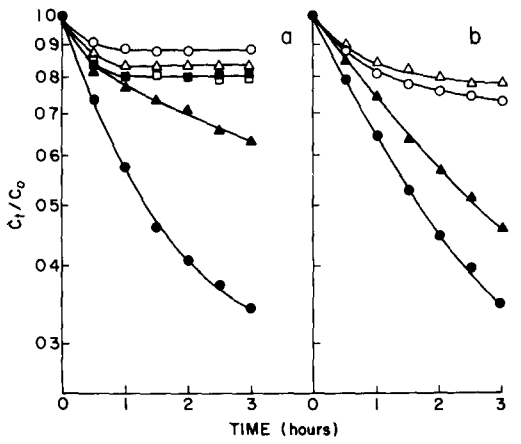


Fig 5 The effects of lanthanum ions on ^{45}Ca efflux in yeast cells Medium contained 20 mM Hepes buffer (pH 5 2) and the following: (a) (■) none, (□) 100 μM $LaCl_3$, (●) 2 mM $MgCl_2$, (○) 2 mM $MgCl_2$ + 100 μM $LaCl_3$, (▲) 2 mM $CaCl_2$, (△) 2 mM $CaCl_2$ + 100 μM $LaCl_3$ (b). (●) 100 mM KCl, (○) 100 mM KCl + 100 μM $LaCl_3$, (▲) 100 mM NaCl; (△) 100 mM NaCl + 100 μM $LaCl_3$

In the experiments shown in Fig. 4, we investigated whether the process of calcium efflux induced by monovalent cations depends on cellular energy. In the absence of glucose in the medium, the efflux of calcium was almost completely inhibited. Antimycin A, a respiratory inhibitor, blocked calcium efflux completely in the absence of glucose. In the presence of glucose the inhibition by antimycin A was very slight since the energy was supplied by fermentation. We conclude that the activation of calcium efflux by monovalent cations is an energy-dependent process.

It was reported recently that in yeast cells lanthanum ions are an efficient inhibitor for the uptake of calcium [8]. We have therefore examined the effect of lanthanum on calcium efflux (Fig. 5). Lanthanum chloride (100 μM) had no effect on the efflux of calcium in the absence of ions in the medium, but inhibited almost completely the efflux of calcium induced by the magnesium and to a lesser extent the efflux of calcium induced by monovalent cations.

In order to determine whether the activation of calcium efflux by monovalent cations is accompanied by the influx of these ions into the cells, we have measured the ionic content of yeast cells after incubation in Hepes-glucose medium containing KCl or NaCl. The results in Table III indicate that

TABLE III

THE AMOUNT OF CATIONS IN YEAST CELLS AFTER INCUBATION IN DIFFERENT MEDIA

Hepes-glucose medium contained 20 mM Hepes (pH 5.2) and 0.1 M glucose Cells were incubated in the above media for 24 h

Medium	Cations in cells (nmol/mg dry weight)		
	Ca^{2+}	K^+	Na^+
Medium I	2 5 ±0 1	382 1±19 2	118 9±17 2
Hepes-glucose + 0.1 M KCl	0 17±0 05	578 3±22 0	24 5± 1 7
Hepes-glucose + 0 1 M NaCl	0.36±0 2	45 7± 3 1	324 8±23 1

the efflux of calcium in the presence of K^+ or Na^+ is accompanied by influx of those cations into the cells.

Discussion

Whereas the pathway of uptake of divalent cations in yeast cells is well defined [7–9,11,12,16] there is only little information available on the pathway of efflux of the cations. It was long accepted in yeast cells that divalent cations are taken up into non-exchangeable pools [7]. However, it has recently been shown that calcium efflux can occur [12]. In our previous work we have found that in the absence of cations in the medium only 20% of cellular calcium left the cells during the first 2 h. There was no additional efflux of calcium during 25 h after the initial outflow. In the presence of magnesium or calcium in the medium substantial efflux occurred. The rates of this efflux were dependent on the concentrations of the divalent cations in the medium [1]. These results led to the suggestion that calcium-efflux carriers require loading with divalent cations on either side for transport to occur; i.e. only Ca^{2+}/Ca^{2+} or Ca^{2+}/Mg^{2+} exchange can occur.

In this study we found that the presence of monovalent cations in the medium also activates calcium efflux system in a concentration dependent manner. However, the specific effects of monovalent cations on the parameters of calcium efflux were different from those of the divalent cations. The presence of Na^+ or K^+ in the medium, apart from an activation of the efflux of calcium from the 'slow-turnover' calcium pool, also increased the proportion of cellular calcium in the 'fast-turnover' calcium pool. These results introduce some difficulties in the localization of the 'pools' to specific cellular organels, as was done in mammalian cells. In several types of mammalian cells it was suggested that the 'fast-turnover' calcium pool represented the cytosolic calcium and the 'slow-turnover' calcium pool represented the mitochondrial calcium [17,18–20]. In yeast cells the change in calcium distribution between the pools by an increase in the concentration of monovalent cations in the medium may lead to the suggestion that each of the pools is composed of several subpools which are activated by different factors. An alternative interpretation may be that the influx of cations actually changes the distribution of calcium between the pools. In this case, the 'fast-turnover' calcium pools probably represent the cytoplasmic calcium and the 'slow-turnover' calcium pools may represent calcium in the mitochondria, vacuoles or both. Both of these organelles have been shown to contain divalent cations [21–23].

The stimulation of calcium efflux was accompanied by the influx of K^+ or Na^+ into the cells. These results suggest that the activation of calcium efflux was due to Ca^{2+}/K^+ or Ca^{2+}/Na^+ exchange. In the literature, some indications can be found for exchange between divalent and monovalent cations in yeasts: Influx of magnesium, manganese, cobalt and cadmium were accompanied by efflux of potassium [10,11]. It has also been postulated that uptake of calcium took place in exchange for intracellular potassium [24]. Some support for K^+/Ca^{2+} exchange could be deduced also from the fact that substrates that induce efflux of K^+ from the cells caused an increase in calcium uptake [13]. In mammalian cells, the occurrence of Ca^{2+}/Na^+ exchange is well established [25]. In all the above experiments in yeast, as well as in the present study, it was not determined whether the monovalent-divalent cation exchange was mediated by electrically-coupled independent carriers or by non electrogenic carriers which require loading of cations on both sides (divalent cation/ monovalent cation antiport).

The finding that activation of calcium efflux by monovalent and divalent cations involves changes in different parameters indicates that the two processes are mediated by different mechanisms. Additional support for this notion can be derived from the experiments depicted in Fig. 3. The activation of calcium efflux by NaCl showed saturation at 50 mM NaCl since increasing the concentration to 100 mM did not exert an additional effect. However, addition of 2 mM $MgCl_2$ to 100 mM NaCl caused additional changes in the parameters of calcium efflux: an increase in the rates of efflux from both calcium pools. These results suggest that the activations of calcium efflux by monovalent and divalent cations were mediated by different mechanisms.

The experiments with Antimycin A, the respiratory inhibitor, showed that both processes were energy dependent, and could utilize energy either from fermentation or from respiration (Fig. 4 and Ref. 1).

Further experiments to characterize the activation of calcium efflux by monovalent cations were carried out using $LaCl_3$. Lanthanum ions were found to be one of the most effective inhibitors of calcium influx in yeast [8]. In some types of mammalian cells, lanthanum ions were also found to inhibit calcium efflux [26]. The results of the present study indicate that in yeast, lanthanum did not inhibit the small efflux of calcium into medium without cations, but completely inhibited the stimulation of calcium efflux by magnesium and to somewhat lesser extent by calcium. This inhibition was probably due to the inhibition of magnesium or calcium influx by lanthanum; it is established that in yeasts, calcium and magnesium share the same carrier system [8]. The inhibition of calcium efflux by lanthanum in the presence of K^+ or Na^+ is more difficult to interpret. Lanthanum ions were found to inhibit Rb^+ uptake in yeasts [28]. However, since the V_{max} of Rb^+ uptake was not affected by La^{3+} [28], one would not expect any inhibitory effect on K^+ or Na^+ uptake at the high concentrations of these cations used in the experiment (100 mM). Therefore, it is reasonable to suggest that the component of calcium efflux activated by cations is directly inhibited by lanthanum.

In conclusion: We have described the activation of calcium efflux by monovalent cations, determined its parameters and some of its characteristics. More work is required to understand the mechanism of the process.

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