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A putative K^+-selective channel in the plasma membrane of yeast that is blocked by micromolar concentrations of external divalent cations and is insensitive to tetraethylammonium

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At pH 7, addition of glucose to an anaerobic suspension of non-metabolizing yeast cells causes a transient net efflux of K^+ from the cells and a concomitant transient hyperpolarization of the plasma membrane (Van de Mortel, J.B.J., et al. (1988) Biochim. Biophys. Acta 936, 421–428). Both phenomena are effectively suppressed in the presence of low concentrations of polyvalent cations. The concentrations of Mn^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Sr^{2+} and La^{3+} required for half-maximal suppression of the transient hyperpolarization are 10, 17, 20, 38, 47 and 5 μM, respectively. Subsequent addition of EDTA 90 s after that of Ca^{2+} immediately restores both K^+ efflux and cellular uptake of the fluorescent membrane potential probe 2-(dimethylaminostyryl)-1-ethylpyridinium (DMP). This sugggests that an interaction of polyvalent cations with an external binding site blocks the putative K^+-selective channel. Opening of this channel is not blocked by 20 mM tetraethylammonium nor by 100 μM 3,4-diaminopyridine. It is argued that this glucose-induced K^+-conductive pathway is not identical to the voltage-gated K^+ channels identified until now in patch-clamp studies of the yeast plasma membrane.

Introduction

Previously we showed that addition of glucose to non-metabolizing *Saccharomyces cerevisiae* cells induces a transient net outflow of K^+ from the cells and a concomitant hyperpolarization of the plasma membrane [1]. The phenomenon is only seen at pH values above 5.5. It was hypothesized that a yet unknown event occurring at the onset of metabolism, possibly related to increased cAMP synthesis triggers the opening of an ion-conductive pathway in the plasma membrane of the yeast cells. The finding that 1.25 mM K^+ completely abolishes the hyperpolarization, whereas the same concentration of Na^+ has no effect at all, indicates that the pathway is K^+ selective. A quantitative analysis of the effect of K^+ on the membrane potential furthermore revealed that the hyperpolarized cells behave by approximation as K^+ electrodes, which is compatible with the notion that during hyperpolarization, specific K^+-conducting channels are opened.

Patch-clamp studies have demonstrated already before that also yeast cells possess voltage-gated K^+-selective channels in their plasma membranes. Gustin et al. [2] showed that these channels have properties of a high selectivity for K^+ over Na^+, unit conductance of 20 pS, inhibition by millimolar concentrations of TEA or Ba^{2+} and bursting kinetics. Recently K^+ channels have been identified in a plasma membrane H^+-ATPase mutant of *Saccharomyces cerevisiae* of altered voltage dependence which are sensitive to ATP and inhibitors of the H^+-ATPase [3]. Apparently, a mutation in the gene that codes for that plasma membrane ATPase affects the gating of this voltage-dependent K^+ channel.

Whether the K^+-selective pathway opened at the onset of metabolism is an identical entity as the voltage-gated K^+ channel identified in the patch-clamp experiments quoted above is examined in the present paper. Use is made of the fact that the latter K^+-conductive pathway is blocked by external TEA or Ba^{2+} and not by Ca^{2+}, even when applied in millimolar concentrations [2]. We will show that 20 mM TEA does not prevent the glucose-induced transient K^+ efflux

Abbreviations: DMP, 2-(dimethylaminostyryl)-1-ethylpyridinium; TEA, tetraethylammonium; EDTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; DAP, 3,4-diaminopyridine.

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and hyperpolarization. Furthermore, besides Ba^{2+}, also various other divalent cations, notably Ca^{2+}, block the transient K^+ efflux and the hyperpolarization already at micromolar concentrations without profound ion selectivity. The putative glucose-induced K^+-selective channels might therefore represent an other class of K^+-selective channels that remained unobserved in patch-clamp experiments conducted so far and play a role in a nutrient-induced signal transmission pathway of yeast cells [4].

Materials and Methods

10% (w/v) *S. cerevisiae* (Koningsgist) was starved for 24 h in distilled water by aeration at room temperature. After the cells were exhausted, they were washed twice with distilled water and once with buffer (45 mM Tris brought to pH 7.0 with succinic acid). Then the cells were suspended in buffer at a density of 10% (w/v).

Uptake of the fluorescent membrane potential probe DMP was determined according to Ref. 1. As a measure for the rate of influx of DMP we took the maximal slope of the curves obtained. Transiently hyperpolarizing yeast was obtained by adding glucose (5% w/v) to the cell suspension (2% w/v) of non-metabolizing cells placed in a multi-purpose cuvet. Maximal hyperpolarization was observed after about 90 s. Cells with a 'normal' membrane potential were obtained by preincubating the cells for 30 min in the presence of glucose before the addition of DMP. The cell suspension was bubbled through with N_2. Then the cell suspension was transferred to the multi-purpose cuvet and DMP was added. The cell suspension in the multi-purpose cuvet was not bubbled through with N_2 but mechanically stirred. The K^+ concentration in the medium was registrated continuously according to Ref. 1 in parallel experiments. The cell suspensions were bubbled through with N_2.

All chemicals used were of analytical grade. DMP and DAP were purchased from Sigma, St. Louis, MO, U.S.A. The yeast was kindly provided by Gist-Brocades, Delft, The Netherlands.

Results

Addition of glucose to an anaerobic suspension of yeast cells at pH 7 triggered with a lag period of approx. 40 s the uptake of the fluorescent membrane potential probe DMP into the cells (Fig. 1). After 10 min the fluorescence intensity decreased again [1], which means that after an initial hyperpolarization the plasma membrane became partially depolarized. Various divalent cations added together with the glucose at zero time strongly reduced the rate and amplitude of the increase in DMP fluorescence. This shows that already micromolar concentrations of divalent cations are able to suppress the transient hyperpolarization of the yeast cell membrane induced by glucose.

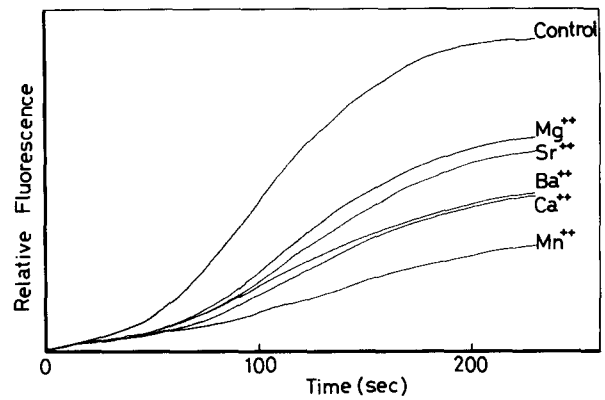

Fig. 1. Effect of divalent cations upon the glucose-induced fluorescence intensity increase of DMP at pH 7. To a suspension of 2% (w/v) non-metabolizing yeast 40 μM of various divalent cations were added simultaneously with 5% (w/v) glucose and 1.4 μM DMP at zero time. The tracings are representative of three experiments done in duplicate.

Fig. 2 shows for various divalent cations the concentration dependence of their effect on the transient hyperpolarization. In this figure the percentual decrease in maximal rates of the increase in DMP-fluorescence intensity, are plotted against the divalent cation concentration (Fig. 2A). Fig. 2B represents Eady-Hofstee plots of the data and shows that the suppression of the transient hyperpolarization by the divalent cations exhibits saturation kinetics, which can be described by a Michaelis-Menten equation with a maximal inhibition amounting to 90%. The concentrations of divalent cations required for half-maximal effects are 10, 17, 20, 38, and 47 μM for Mn^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+} and Sr^{2+},

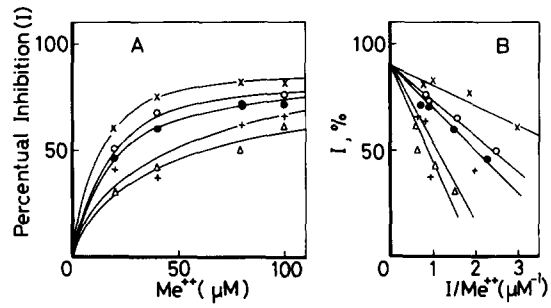

Fig. 2. Concentration dependence of the suppression of the glucose-induced DMP uptake into yeast cells by various divalent cations. In A the percentual decrease in the maximal rate of the DMP fluorescence increase (I) is plotted against the divalent cation concentration Me^{2+} (μM). In B the data are represented according to an Eady-Hofstee plot [5]. For a relation between I and Me^{2+} obeying Michaelis-Menten kinetics, straight lines are obtained according to this way of plotting: the intercept of the line with the ordinate represents the maximal percentual decrease in the rate of DMP uptake, its slope the concentration of the divalent cation required for half-maximal effect. Each point represents the mean value of triplicates. (×) Mn^{2+}, (○) Ca^{2+}, (●) Ba^{2+}, (+) Mg^{2+} and (△) Sr^{2+}.

respectively. The trivalent cation La^{3+} appeared to suppress the transient hyperpolarization more effectively than the divalent cations applied. It exerted its half-maximal effect already at a concentration of 5 μM (data not shown).

Under those conditions where the cells were no longer hyperpolarized, i.e., 30 min after the addition of glucose to the non-metabolizing cells [1], addition of the divalent cations or La^{3+} at concentrations that completely blocked the transient hyperpolarization had no effect anymore upon the rate of DMP uptake (data not shown).

As in our view the transient hyperpolarization is caused by a transient opening of K^+-selective channels in the plasma membrane, it is expected that polyvalent cations which suppress the hyperpolarization should reduce the concomitant K^+ efflux, as well. Fig. 3 shows that this is indeed the case. In the absence of added polyvalent cations, K^+ efflux was relatively large. Addition of 40 μM of divalent cations together with the glucose greatly reduced this efflux. In the presence of those divalent cations which gave the strongest suppression of the glucose-induced hyperpolarization (Mn^{2+}, Ca^{2+} and Ba^{2+}, see Fig. 1) a small but significant net uptake of K^+ occurred during the first minutes. Upon longer incubation this influx reversed into a net transient efflux which remained much smaller than the one was observed in the control or in the presence of Sr^{2+} or Mg^{2+}.

Comparable to their ability to suppress the transient hyperpolarization, Sr^{2+} and Mg^{2+} were less effective than Mn^{2+}, Ca^{2+} and Ba^{2+} in preventing the glucose-induced net outflow of K^+ ions from the cells, and

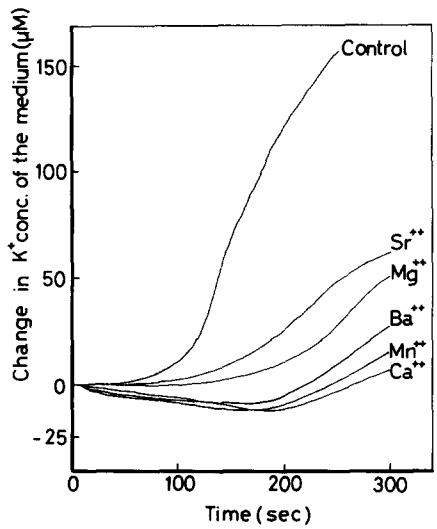

Fig. 3. Effect of divalent cations upon the glucose-induced efflux of cellular K^+ ions. The time courses of changes in the K^+ concentrations in the medium relative to that before the addition of glucose to the non-metabolizing cells (53 ± 8 μM K^+) at zero time were continuously registrated with a K^+-sensitive electrode. See also the legend to Fig. 1.

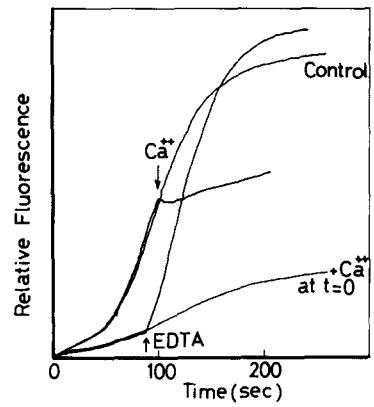

Fig. 4 Effect of Ca^{2+} upon the time course of glucose-induced changes in DMP fluorescence. Comparison of effects of 80 μM Ca^{2+} added at zero time and 90 sec after the addition of 5% (w/v) glucose to the non-metabolizing yeast cells. In addition the effect of complexation of external Ca^{2+} by addition of 80 μM Na-EDTA at the time indicated is shown. See also the legend to Fig. 1.

La^{3+} was more effective than any of these divalent cations (data not shown).

Gustin et al. [2], showed that the plasma membrane of yeast cells possesses voltage-activated K^+-selective channels that can be blocked by 20 mM external TEA, a classic inhibitor of K^+ channels in numerous tissues [6]. TEA, used by us at the same concentration did neither affect the K^+ efflux nor the transient hyperpolarization induced by glucose. Also the more potent K^+-channel blocker, 3,4-diaminopyridine [7] was even at a concentration of 100 μM without effect (data not shown).

In Fig. 4 we show the effect of Ca^{2+} addition to the cells during the glucose-induced hyperpolarization. Addition of 80 μM Ca^{2+} at 90 s after the addition of glucose (the time at which the hyperpolarization is maximal, as could be judged from the dependence of the influx rate of DMP upon the preincubation time with glucose [1]), almost immediately impaired the DMP uptake. On the other hand, the suppression of the DMP uptake by Ca^{2+} added together with glucose at zero

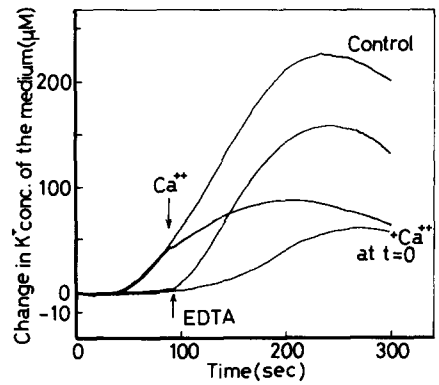

Fig. 5. Effect of Ca^{2+} upon glucose-induced changes in the K^+ concentration of the medium. See also the legend to Fig. 4.

time, was almost immediately relieved upon complexation of the Ca^{2+} ions in the medium by adding EDTA to the cell suspension at 90 s.

Apparently, binding of Ca^{2+} to an external binding site of the channel causes a reversible blockage of its K^+ conductance. In agreement with this notion, addition of Ca^{2+} during the glucose-induced hyperpolarization stopped the K^+ efflux from the cells also almost immediately, the blockage of this efflux being relieved again upon addition of EDTA (Fig. 5). Similar effects of EDTA on the suppression of the glucose-induced hyperpolarization and K^+ efflux by 8 μM La^{3+} were observed (data not shown).

Discussion

The purpose of the present study was to further characterize the putative K^+-selective channel in the plasma membrane of yeast, which transiently opens at the onset of metabolism of the cells at pH values above 5.5 [1].

We have found that micromolar concentrations of various polyvalent cations suppress two processes which are a direct consequence of the opening of this class of K^+-selective channels; hyperpolarization of the cells and a concomitant efflux of K^+ ions [1]. The finding that, e.g., Ba^{2+} and Ca^{2+} apparently block the channel already at micromolar concentrations with only little ion specificity distinguishes this class of K^+-selective channels from those identified by others in patch-clamp studies of the yeast plasma membrane (see Ref. 8 for review). The K^+-selective channels demonstrated in these patch-clamp experiments are namely selectively blocked by millimolar concentrations of Ba^{2+} and not by Ca^{2+}, and are sensitive to TEA [2], while those opened at the onset of metabolism are insensitive to this classical inhibitor of K^+-selective channels. Also DAP, which has been claimed to be a more potent and selective blocking agent of K^+-selective channels than TEA [7] does not block the putative K^+ channel found by us. Apparently the plasma membrane of yeast possesses at least two classes of K^+-selective channels which might differ in gating properties. The class of K^+-selective channels demonstrated in patch-clamp experiments is voltage-gated [23]. A second class of channels, opened at the onset of metabolism might be second-messenger operated. As the addition of glucose to the yeast cells causes a transient increase in cellular cAMP (see, e.g., Ref. 4; Van de Mortel, unpublished results), it is tempting to speculate that cAMP fulfills a second messenger function in the gating of this class of channels. The possibility that the suppression of the glucose-induced hyperpolarization by divalent cations should be ascribed to a depolarization of the membrane instead of to the blockage of the K^+-selective channel has also been considered. Since after 30 min incubation of the cells with glucose, at which condition the membrane potential has its normal value [1] the divalent cations do not appreciably affect the membrane potential, a depolarization of the membrane by divalent cations is only feasible if, shortly after the addition of glucose, also the permeability of the plasma membrane to these cations is transiently increased. However, there are indications (Belde, P., et al., unpublished results) that under conditions that the membrane becomes transiently hyperpolarized, its permeability for Ca^{2+} is just decreased instead of increased. Furthermore, addition of divalent cations under those conditions, leads to a decrease in the efflux of K^+ (instead of to its stimulation, which would be expected if the divalent cations depolarize the cell membrane).

So it seems more likely that the divalent cations cause a blockage of the K^+ channels. In this respect divalent cations have a similar effect as acidification of the medium, which also causes a strong suppression of both the DMP uptake and the K^+ efflux [1]. We therefore, suggest, that the K^+-selective channels which are opened at the onset of metabolism, can be blocked by the binding of polyvalent cations to the negatively charged external sites of the channels or by protonation of these sites. The apparent pK_a of these sites can be deduced from data on the effect of pH on the DMP uptake published earlier [1] and is approx. 6.8.

The almost immediate impairment of the glucose-induced hyperpolarization and concomitant K^+ efflux by Ca^{2+} and the immediate relieve of the suppression of both glucose-induced phenomena by Ca^{2+} upon its complexation with EDTA supports the notion that blockage of the channel by polyvalent cations results from an interaction with an external site on the channel.

The suppression of the transient hyperpolarization by polyvalent cations decreases in the order $La^{3+} > Mn^{2+} > Ca^{2+} > Ba^{2+} > Mg^{2+} > Sr^{2+}$. This order of selectivity is by approximation the same for the effects of the polyvalent cations on the K^+ efflux, the differences between Mn^{2+}, Ca^{2+} and Ba^{2+}, however, are much less clear. This may be partly due to interference of the divalent cations with K^+ uptake, which uptake apparently also occurs during the transient hyperpolarization and may show a different order of sensitivity to the polyvalent cations than the efflux of K^+ through the K^+ channel.

In summary, the work presented here shows that yeast cells possess in their plasma membrane a K^+-selective channel, which opening at the onset of metabolism can be effectively blocked by micromolar concentrations of external polyvalent cations. This distinguishes this channel from those discovered by others in patch-clamp experiments [2,3] and may therefore represent another class of K^+-selective channels. Its insensitivity to TEA and 3,4-diaminopyridine also supports that notion.

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