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Simulation of all-or-none K^+ efflux from yeast provoked by xenobiotics

G.W.F.H. Borst-Pauwels

Laboratory of Chemical Cytology, Roman Catholic University, Toernooiveld, Nijmegen (The Netherlands)

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In experiments dealing with the effect of xenobiotics upon the efflux of K^+ from yeast cells, one should be aware that when this efflux proceeds via an all-or-none process, the K^+ being released from the intoxicated cells can again be accumulated into the still unaffected cells. Therefore, the measured net efflux of K^+ will be less than the efflux from the intoxicated cells. The difference between these two magnitudes can be minimalized by incubating the cells for only a short period and on applying yeast densities that are not too high. When the cells are permeabilized relatively slowly but ultimately to a great extent, the kinetics of K^+ efflux may be quite complicated.

Introduction

A large number of toxic compounds give rise to efflux of K^+ from yeast cells. This applies to detergents [1–3], organic dyes [3–5], inhibitors of plasma-membrane ATPase including calmidazolium antagonists [6–8], polycations [9–10], and heavy metal ions [11–13]. Most of these compounds act by means of an all-or-none process [1–4,9,14–16]. This means that during interaction of the toxic compound with the yeast cell, a gradually increasing number of cells become leaky for K^+, giving rise to a nearly complete loss of K^+ from these cells. The K^+ being lost from these cells, however, can be accumulated again by the cells that are still intact. Therefore, the K^+ efflux from the intoxicated cells will be underestimated. We have now carried out a model study of the appearance of K^+ into the medium when the cells become leaky according to an all-or-none process. This study may contribute to a better understanding of the kinetics of K^+ efflux provoked by toxic compounds in yeast cells.

Theory

K^+ uptake in yeast proceeds by means of a two-site carrier [17]. The rate equation consists of a quadratic equation with respect to the K^+ concentration in the medium (S_e). However, at not too low K^+ concentration at which one of the two binding sites is already saturated with K^+, the rate equation approximate:

$$V_i = \frac{V_m \cdot S_e}{K_m + S_e} \quad (1)$$

K_m is the concentration (in mM) at which the rate of uptake is half-maximal, and V_m is the maximum rate of uptake expected when the carrier is saturated with the cation. V_m is expressed in mmol $\cdot$ kg^{-1} (dry weight) per min. V_m still depends upon the internal K^+ content, S_i. Increasing S_i leads to a feedback inhibition of the uptake of K^+ into the

Correspondence: G.W.F.H. Borst-Pauwels, Laboratory of Chemical Cytology, Faculty of Science of the Roman Catholic University, Toernooiveld, 6525 ED Nijmegen, The Netherlands.

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cells [18]. This is also found for the K^+ uptake in plant roots [19]. Though the mechanism of this feedback is still a matter of discussion, the dependence of the influx rate upon S_i can be described very well by the following equation based upon a still hypothetical allosteric model of the K^+ carrier having n binding sites facing the cellular side of the plasma membrane [19].

$$V_m = \frac{V_0 \cdot S_i^{-n}}{K_i^{-n} + S_i^{-n}} \quad (2)$$

V_0 is the maximum rate of uptake when S_i approximates to zero. K_i is defined by $K_i^n = S_i^n \cdot C/S_{i_n}C$; in which $S_{i_n}C$ is the cell content of the complex between carrier and cellular K^+, and C is the amount of carrier which is not occupied by cellular K^+. For the strain of *Saccharomyces cerevisiae* Delft 2, $n = 16$ and $K_i = 511$ mmol $\cdot$ kg^{-1}. These two values are calculated from the data given in Ref. 20. V_0 is taken as 22.5 mmol $\cdot$ min^{-1} $\cdot$ kg^{-1}. The K_m is taken as 0.5 mM, which is the K_m for K^+ uptake at pH 4.5 [17]. The rate of efflux of K^+ from intact cells is assumed to be linearly related to S_i by

$$V_e = R \cdot S_i \quad (3)$$

From the rate of K^+ efflux from non-metabolizing cells, we obtained a value of $R = 0.003$ mmol $\cdot$ kg^{-1} $\cdot$ min^{-1} at an external pH of 4.5 in 45 mM Tris-succinate buffer under anaerobic conditions. Approximately the same value was obtained for metabolizing cells at pH 4.5 from the rate of efflux of Rb^+ from cells, which were preloaded with carrier-free ^{86}Rb, and on assuming that Rb^+ is released at the same rate as K^+ [21]. Both V_0 and V_e are expressed in mmol $\cdot$ kg^{-1} $\cdot$ min^{-1}. By taking $V_i = V_e$, the expected steady-state concentrations of K^+ both inside and outside the cell can be calculated for a given initial cellular K^+ content. This steady state will be established after adding the yeast cells to a medium being free of K^+.

Addition of a toxic compound giving rise to an all-or-none release of K^+ from the intact cells will lead to a gradual decrease in the fraction of cells which are still intact (F). It is assumed that F is given by:

$$F = A + (1 - A) \cdot \exp(-B \cdot t) \quad (4)$$

A can vary from 1 to 0. At $A = 1$, no breakdown of the K^+ permeability of the plasma membrane occurs, and at $A = 0$, all cells become gradually permeabilized, whereas at intermediate values of A, only a fraction of cells is permeabilized. B is a constant determining the rate of exponential breakdown of the cell membranes. The rate of K^+ efflux due to permeabilization of cells is given by:

$$V_p = -F' \cdot S_i \quad (5)$$

F' is the first derivative of F to the time of incubation of the cells with the toxic compound. For the calculation of the total net flux of K^+ in the presence of the toxic compound, one should account for the decrease in the fraction of still intact cells. The net flux per unit of volume of the cell suspension is given by:

$$V_n = (V_e \cdot F - V_i \cdot F + V_p) \cdot Dw \quad (6)$$

$Dw = 0.6\ V_2$ is the dry weight of the yeast per unit of volume of the yeast suspension at zero time. V_2 is a measure for the cell density representing the ratio of the total volume of cell water (which is approximately half the volume of the cells) at zero time and the volume of the cell suspension.

The changes in the concentration of K^+ in the medium caused by the addition of a toxic compound to the cell suspension are calculated by means of numerical integration on an ATARI 1040 microcomputer. The program is written in Pascal using a CCD Pascal + compiler.

Results

Fig. 1 shows a comparison between the kinetics of K^+ efflux and the time-course of the appearance of cells which have lost all their K^+. The value of A is taken as 0.5, which means that at maximum 50% of the cells become permeabilized for K^+. Two values of B (see Eqn. 4) have been applied. At $B = 1.0$, the cells are rapidly permeabilized for K^+. This leads to a rapid efflux of K^+ amounting at maximum to 42% of the initial K^+ content of the cells at 3 min. After reaching this maximum, K^+ is again taken up by the still intact cells, and the amount of K^+ being released is decreased to 26% at 30 min. At $B = 0.1$, no re-uptake of K^+ occurs. The relative efflux of K^+

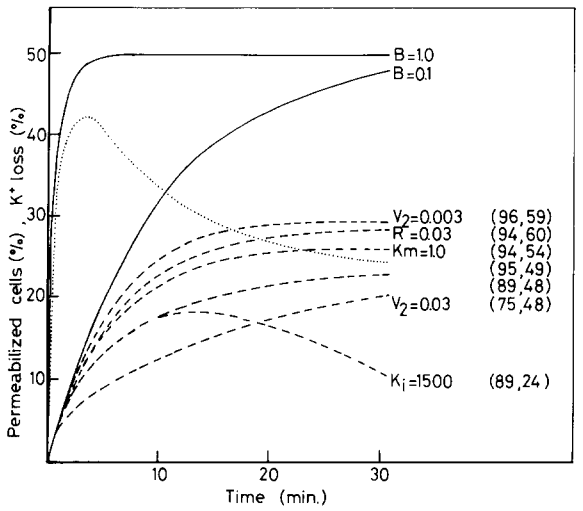

Fig. 1. Calculated percentage of cell K^+ released to the medium and the percentage of cells which are permeabilized for K^+ under conditions that at maximum 50% of the cells are permeabilized ($A = 0.5$) by addition of a compound causing an all-or-none breakdown of the cell membrane. Comparison of various possible experimental conditions was made with regard to the yeast cell density (V_2), the kinetical parameters of K^+ uptake (R and K_m) and the rate of interaction of the xenobiotic with the yeast cell membrane (B). The values of B are indicated in the figure. Standard conditions are $K_m = 0.5$ mM; $K_i = 511$ mmol·kg^{-1}; $R = 0.003$ mmol·kg^{-1}·min^{-1}; $V_0 = 22.5$ mmol·kg^{-1}·min^{-1}; and $V_2 = 0.01$. Deviations from this standard condition are indicated in the figure. ——— represents the percentage of cells which are permeabilized. Percentage of K^+ being lost to the medium is indicated by ····· or - - - - - - at $B = 1$ and $B = 0.1$, respectively. The two figures between parentheses are the percentages of K^+ relesed to the medium taking the amount of K^+ which would be released when no re-uptake occurs as 100% after 1 min and 30 min incubation of the cells with the xenobiotic, respectively.

is decreased on increasing the yeast density from 0.01 to 0.03, and is increased at $V_2 = 0.003$. An increase in the K_m from 0.5 to 1 mM, or an increase in the efflux rate constant from 0.002 to 0.02 mmol · kg^{-1} · min^{-1} leads to a higher amount of K^+ being released. By increasing K_i to 1.5 mol · kg^{-1}, a condition is simulated in which hardly any restriction upon the maximal rate of K^+ uptake is imposed. As expected, this leads to extensive re-uptake of K^+. After 120 min, almost all K^+ being initially released is again accumulated into the cells (data not shown). We have also indicated in Fig. 1 the percentages of K^+ released to the medium taking the amount of K^+ which would be released when no re-uptake occurs as 100% after both 1 min and 30 min incubation with the xenobiotic. Apparently, after 1 min incubation, there is only a small discrepancy between the values expected when no re-entry occurs and the values found when re-entry is accounted for. On the other hand, the amount of K^+ being released after 30 min incubation is considerably less than the amount expected when no re-entry occurs.

Fig. 2 shows that at $A = 0.1$ (90% of the cells become ultimately leaky for K^+) much more K^+ is released than when $A = 0.5$. If the rate by which the cells are permeabilized is relatively low ($B = 0.02$), the kinetics of K^+ release become rather complicated. At first, there is a rapid efflux of K^+ followed by a slow efflux which again is followed by a rapid efflux, which finally again levels off. This is not found anymore when B is increased to 0.05. Also in these cases, the differences between the amount of K^+ released after 1 min incubation of the cells with the xenobiotic and that when accounting for re-entry of K^+ and the amount of K^+ expected when no re-entry occurs, is small.

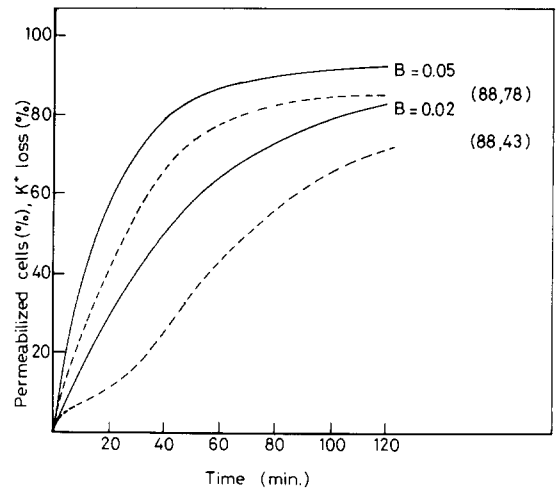

Fig. 2. Calculated percentage of cell K^+ released to the medium and the percentage of cells which are permeabilized for K^+ after addition of a compound causing at maximum 90% of the cells to become leaky for K^+ at varying rates of interaction of the compound with the yeast cell. $A = 0.1$; $V_2 = 0.01$; $K_m = 0.5$ mM; $K_i = 511$ mmol·kg^{-1}; $R = 0.003$ mmol·kg^{-1}·min^{-1}; and $V_0 = 22.5$ mmol·kg^{-1}·min^{-1}. The values of B are indicated in the figure. See subscript to Fig. 1 for further details.

Discussion

As pointed out in the introduction, the appearance of K^+ into the medium is frequently used as a measure for the toxic action of xenobiotics upon yeast cells. Our study shows that one should be cautious with the interpretation of that type of result.

The time-course of appearance of K^+ into the medium can be rather complicated if the K^+ loss is caused by an all-or-none interaction of a poison with the cells. The efflux of K^+ from the cells which have become leaky for K^+ will be counteracted by uptake of K^+ into the still intact cells. This can lead to various types of K^+ efflux curves. Besides 'normal' smooth curves as seen in Fig. 1, it is also possible that the efflux turns gradually into a subsequent uptake or that the net efflux rate of K^+ shows oscillations (see Fig. 2). The latter is only expected when permeabilization of the cells proceeds relatively slowly (low B value) and the final extent of permeabilization is high (low value of A). Experimentally, re-uptake of K^+ has been found on adding calmidazolium to metabolizing cells, whereas with non-metabolizing cells, which do not accumulate K^+, no re-uptake was found [8]. Also ethidium added at relatively low concentrations to a cell suspension of metabolizing cells caused, after an initial release of K^+, re-uptake of this cation [16,22]. This was also found for DIO-9 (Van de Mortel, J., unpublished data). In all these cases re-uptake was limited. This is in accordance with our model, because, due to the feedback mechanism of K^+ uptake, only a limited amount of K^+ can enter the still intact cells. Sigmoidal curves for K^+ efflux have been found with various K^+ efflux provoking agents [23]. Whether these represent examples of the kinetics depicted in Fig .2 or whether these sigmoidal curves are due to the fact that the poison is not immediately interfering with the yeast cell (e.g., before it should first accumulate into the cells) is not clear at this stage.

The best way to diminish the complications which may arise in the interpretation of the kinetics of K^+ efflux is to take care that the incubation of the cells with the xenobiotics is relatively short and the yeast density is not too high. Figs. 1 and 2 show that for various simulated experimental conditions, efflux of K^+ after 1 min incubation of the cells is still relatively high. Accordingly, we found on incubating 2% w/v (fresh weight) yeast corresponding with $V_2 = 0.01$ with various inhibitors of yeast plasma-membrane ATPase a good correlation between the inhibition of plasma-membrane ATPase and the efflux of K^+ [8]. Also the uptake of Sr^{2+} after 1 min incubation with the xenobiotics, which uptake is stimulated by these compounds, appeared to be well correlated with the efflux of K^+. After longer periods, for example a 30 min incubation, the discrepancy between the expected K^+ efflux due to permeabilization of the cells without re-uptake and the K^+ efflux expected when re-uptake proceeds is rather high.

Comparison of the effect of a poison upon two different strains of yeast cells is only allowed when these cells have the same characteristics for K^+ transport. If this is not true, wrong conclusions may be drawn from the experimental results. As a matter of fact, Theuvenet et al. [23] have found that various organic poisons give rise to a lower efflux of K^+ from a metabolizing Cd-resistant strain of *S. cerevisiae* than from its wild parent strain. Still, the growth of the two yeast strains is affected to the same extent by these poisons. The differences in K^+ efflux are probably due to the fact that the Cd-resistant cells have a much lower rate of K^+ efflux than the wild-type cells, which means that R is decreased during adaptation of the cells towards Cd^{2+}. This view is supported by the fact that the percentage of K^+ being lost at a given concentration of the poison increased more for the Cd-resistant cells than for the wild-type cells on decreasing the density of the yeast suspension.

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