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Energy expenditure induced by changes in the ion homeostasis of wheat root cells

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Abstract

A relationship between energy exchange and ion transport is the basis for the functional response of cells to stress factors. The aim of the present work was to estimate the energy expenditure for response reactions and adaptation that are dependent on the permeability of the plasma membrane of the excised root cells. We studied the dynamics of changes in the (i) energy flow as measured by the oxygen uptake rate and the heat production rate, (ii) the electrical membrane potential (MP) and (iii) the loss of potassium ions of the cells on prolonged (5 h) treatment with membrane active compounds, namely the specific K^+-ionophore valinomycin (Val) and chlorpromazine (CPZ), an antagonist of calmodulin but with a wide spectrum of other action. It was shown that the early (2 h) response of the cells exposed to these compounds was an increase in the loss of K^+ ions and a decrease in the MP that were more pronounced in the presence of CPZ. The rates of oxygen uptake and heat production by the cells in the presence of Val increased with time and were coupled with the restoration of ion homeostasis as measured by the reduced loss of K^+ ions. It is supposed that in the presence of Val the energy dependent recovery of ion homeostasis occurred through the compensatory activation of a plasma membrane H^+-ATPase and the increase of K^+/H^+-exchange. Compared to Val, CPZ had larger effect on the alteration of the membrane characteristics and energy expenditure even during the relatively short time of treatment. It is supposed that this happened because CPZ damaged the membranes of the cells as a result of its partition with membrane lipids. Therefore, more energy is needed to restore the cellular homeostasis in the presence of CPZ compared to Val.

Keywords: Heat production rate; Oxygen uptake rate; Ion homeostasis; Chlorpromazine; Valinomycin; Wheat seedling roots

1. Introduction

The shifts in ion homeostasis due to the changes in the structural and functional properties of the plasma membrane contribute to the regulation of cellular metabolism and the corresponding energy expenditure must encompass these changes. Maintenance of the non-equilibrium distribution of ions between the cells and their environment costs 30% or more of their total energy expenditure [1]. The extent of the disturbance of ion homeostasis caused by different treatments can be influenced by changes in the ratio of the passive to active transport at the plasma membrane. Our previous work [2] showed that during the early response of the excised roots to the specific K^+-ionophore valinomycin (Val) (20 μM), there was only a small loss of potassium ions by the cells and a decrease in the electrical membrane potential (MP) which did not increase with time. Changes in energy metabolism were insignificant, probably because the K^+ loss was so small with this treatment. The membrane-active phenothiazine xenobiotic chlorpromazine hydrochloride (CPZ) at 100 μM caused a significant increase in the K^+ efflux from the roots and a decrease in the cellular MP [2], which increased with time and was accompanied by an inhibition of catabolism as measured by the rates of oxygen uptake to give the respiratory rate and heat production for the total catabolic rate. These results pointed to a reduced energy flow as a result of the damaging effect of CPZ which is a membrane active compound with a wide spectrum of action including modulation of Ca^{2+}/calmodulin dependent processes, membrane ATPases and phosphatases [3–5].

In the present work, an attempt was made to estimate the energy expenditure for the reactions in response to the two effectors and the adaptation by the excised wheat roots, which it

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is supposed is dependent on the plasma membrane permeability [2]. For this purpose, we studied the dynamics of changes in the rates of oxygen uptake and heat production, electrical membrane potential and K^+ ion loss of the cells under the prolonged exposure to Val and CPZ in equimolar concentrations (50 μM).

2. Experimental

The excised roots of 5-day-old seedlings of wheat (*Triticum aestivum* L.) grown in distilled water were the objects of the investigation. The procedure for growing the plants and the details of the methods used were covered comprehensively in our previous paper [2]. Growth of the seedlings and subsequent incubation of the excised roots in distilled water were used to increase sensitivity of roots to applied effectors [6]. The content of K^+ ions released by roots into the incubation solutions for 1, 2, and 5 h was determined using a flame photometer (Phlapho-41, Karl Zeiss, Jena, Germany) [7]. The oxygen uptake rate was registered on an hourly basis by the Warburg manometric method [8]. The excised roots (150 mg) were placed into the 20-ml manometric vessels containing 3 ml of sterile distilled water, which acted as the control or in the experimental solution containing one of the effectors and both sets of vessels were shaken at 100 rev/min with the temperature set at 30 °C. The data are presented as mole $O_2\ h^{-1}$ per g^{-1} fresh weight (f.w.).

The heat production rate was measured by an LKB differential microcalorimeter (LKB-2277 Bio Activity Monitor Bromma, Sweden) as described in [2,9]. Samples of the excised roots (180 mg) were placed in 3 cm^3 glass vessels that were hermetically sealed before thermal equilibration for 30 min. This means that zero time for measuring the heat production corresponded to 30 min after adding the compounds to the roots. The temperature was set at 30 °C. During the experiment the heat power (W), which characterizes the rate of the heat production, was registered and further calculated using following equilibration: $W = \Delta Q/\Delta t$, where Q is the total heat production. Data are presented in mW g^{-1} f.w.

The electrical membrane potential (MP) of root cells was measured using a standard microelectrode technique [10]. Glass microelectrodes were introduced into the cells of the rhizodermis in the root elongation zone, 1.5–2 cm from the root tip. Both of the measurement and the reference microelectrodes were connected to the amplifier by a silver chloride wire via agar-agar bridges.

The membrane active compounds valinomycin (Serva, Germany) and chlorpromazine hydrochloride (Sigma, USA) were used in an equimolar concentration of 50 μM. For the stock solution, 0.06 mg Val was dissolved in 50 μl of ethanol. The corresponding amount of ethanol was added to the distilled water control. The experiments were performed in 3–5 analytical and 3–5 biological replicates.

3. Results

As seen in Table 1, the leakage of K^+ ions in the control was greatest in the first hour of incubation. It gradually reduced thereafter and was not detectable after incubation for 5 h. The CPZ-induced K^+ efflux from the roots was greater than in the control and continued until at least the 2-h measurement. At 5 h the K^+ content in the CPZ medium was less than after incubation for 1 h, i.e. there was a partial re-absorption of the released K^+ in contrast to the total re-adsorption in the control (Table 1).

Table 1
Differences in the mass-specific K^+ content of the incubation medium after exposure of wheat roots to CPZ and Val for various times

	Duration of root incubation (h)	K^+ content in the medium (μequiv./g f.w.)
Control (H_2O)	1	3.9 ± 0.1
	2	0.6 ± 0.1
	5	0.0 ± 0.0
CPZ (50 μM)	1	5.8 ± 0.1
	2	7.3 ± 0.1
	5	4.4 ± 0.1
Val (50 μM)	1	4.9 ± 0.1
	2	5.6 ± 0.0
	5	2.3 ± 0.1

Data are given as mean ± S.D., at $n = 5$.

The CPZ-influenced K^+ loss from the cells at 1 and 2 h was accompanied by a declining decrease in the MP from 30 to 25% (Fig. 1). After exposure of the roots to CPZ for 5 h, there was a rise in the MP coincident with the re-absorption of K^+ ions (see Table 1). By this time, the effect of CPZ had decreased to 19% as seen in Fig. 1.

As can be seen in Fig. 2, CPZ stimulated the rate of oxygen uptake increasingly over the 5-h incubation period (Fig. 2) so that by 5 h the rate was double that of the control and was associated with the increase in the MP and the decrease in the total K^+ lost during the incubation. In contrast to its effect on respiration, CPZ caused a decrease of 30% in the rate of heat production in the initial period (Fig. 3) and was still inhibitory after incubation for 5 h of incubation, although the degree of it was less at 20%.

Turning to the effects of Val, it can be seen in Table 1 and Fig. 1, respectively, that the K^+ loss by the excised roots and the decrease in the MP during the first 2 h were less marked

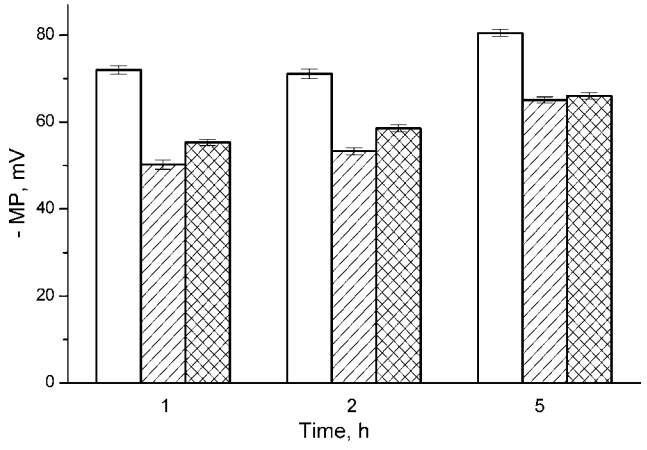

Fig. 1. Effect of 50 μM chlorpromazine (▨) and 50 μM valinomycin (▩) on the membrane potential of the excised root cells, compare to the control (□).

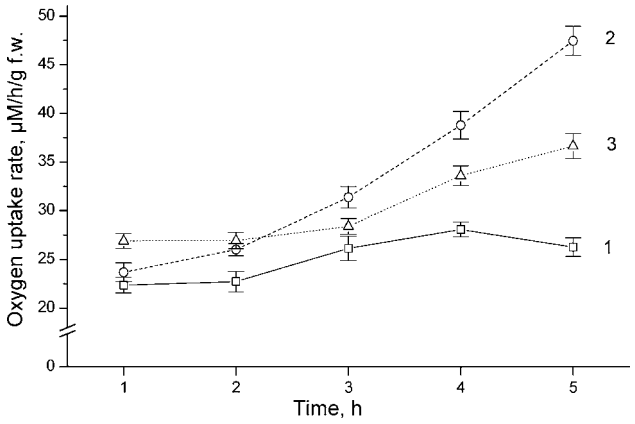

Fig. 2. Effect of 50 μM chlorpromazine and 50 μM valinomycin on the rate of oxygen uptake by the excised roots. 1: control; 2: chlorpromazine; 3: valinomycin.

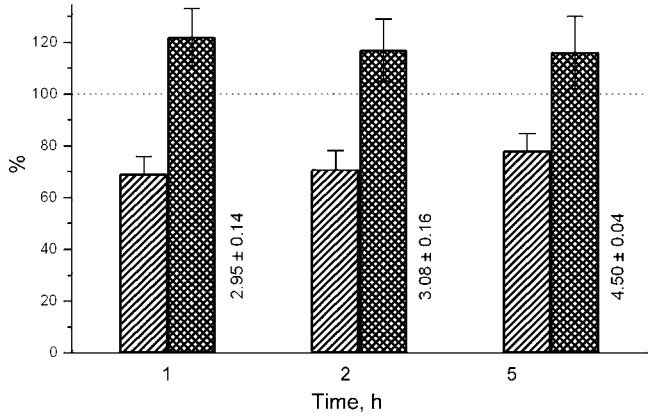

Fig. 3. Rate of heat production by excised roots with chlorpromazine (▨) and valinomycin (▩). Ordinate axis – heat production, %; heat production in control sample was taken as 100%. Numeric values for the control variant are given on the right of the columns in mW g^{-1} f.w.

than with CPZ. After roots had been treated with Val for 5 h, the K^+ content in the incubation medium was less than after 1 h, i.e. there appeared to be a re-absorption of the K^+ ions earlier released by the cells, just as in the control. Similar to the effect of Val for the first 2 h, the K^+ loss by the cells after 5 h was less than that in CPZ for the same time (Table 1). After prolonged treatment of the roots with Val (see Fig. 1), the MP of the cells had increased when compared to the values at 1–2 h and was similar to that after 5 h in CPZ.

Val increased the rate of root oxygen uptake and this became more rapid with time (Fig. 2) but it was less pronounced than in the case of CPZ. The rate of heat production by the roots in the presence of Val, in contrast to those exposed to CPZ was higher by a consistent amount compared to the control for all of the 5-h incubation period (see Fig. 3).

4. Discussion

The early responses of root cells to Val and CPZ was characterized by a decreased MP (Fig. 1) and an increased K^+ loss (Table 1) but they were not affected to the same extent, being more pronounced in the case of CPZ. This result could imply that the latter caused a greater disturbance to ion homeostasis than the former. The changes to the rates of oxygen uptake (Fig. 2) and heat production (Fig. 3) were also different in that the Val-induced increase in plasma membrane K^+ conductivity was associated with the rapid stimulation of the respiratory rate and the total catabolic rate of the cells. It is known that the recovery of ion homeostasis can be coupled with the activation of ATPases of plasma membrane [11]. In animal cells, Na^+,K^+-ATPase plays an important role in the recovery of ion homeostasis, while in plant cells, H^+-ATPase in the plasma membrane is responsible for the recovery of proton gradient. This requires metabolic energy from respiration and involves the changes in K^+ transport and generation of MP. One of the factors involved in regulating H^+-ATPase activity under stress conditions can be K^+ ions [12]. Previously we showed that the increased K^+ leakage after 2 h exposure of roots to Val was accompanied by an increase in the ATPase activity [6].

Our results showing that the intensity of the catabolic processes was more pronounced under the prolonged exposure to Val would appear to be the reason for the reduction in K^+ loss and the increase in the negative MP. We suggest that the cells actively withstood the prolonged Val treatment due to the compensatory activation of an H^+-ATPase which resulted in the enhanced K^+/H^+-exchange. These processes can contribute to the energy dependent regulation of the cytoplasmic pH through the recovery of the membrane proton gradient.

It is suggested that CPZ, because of its multiple effects on the lipid components of plasma membrane, can increase the membrane ion permeability to a greater extent than Val. As a result, CPZ, compared with Val, can cause the greater stimulation of the oxygen consumption during the first 1–3 h of exposure (Fig. 2). Changes in MP (Fig. 1) and K^+ permeability (Table 1) of the CPZ-treated roots over the 5-h period indicate that a partial recovery of cellular ion homeostasis occurred, though it was less than in the case of Val. The concentration of CPZ of 50 μM used in our experiments apparently was low enough to allow at least the partial recovery of the structure, regulation of ion transport and H^+-ATPase activity of the plasma membrane. This is in contrast to Alekseeva et al. [2] who showed that at 100 μM CPZ appeared to cause significant structural damage to the plasma membrane. Los [13] showed that the increase of membrane fluidity triggers gene expression coding the structural proteins of the plasma membrane. They can bind with lipids and prevent the disintegration of the membrane [14]. In addition, the reported CPZ-induced change in the level of Ca^{2+} in the cytoplasm of root cells [2] could alter the balance of lipid synthesis to favour of those lipids with characteristics to maintain membrane microviscosity [15]. Based on these considerations, we suggest that the partial restoration of membrane function in CPZ-treated cells was due to detoxification of the xenobiotic, in particular in the endoplasmic reticulum [16]. Earlier [2] we found that CPZ induced changes to the ultrastructure of this membrane organelle and of mitochondria. These alterations may be part of the response reaction of the cells to the disturbance in their ion homeostasis

and indicate the active role of these organelles in its recovery [17].

Despite the increase in the rate of oxygen consumption induced by CPZ (Fig. 2), the rate of heat production was lower than that in the control (Fig. 3). In animal cells, it is well established that CPZ causes hypothermia and can significantly decrease the temperature of organism [18]. Our results are an indication that similar effect can also happen in plants. The possible lowering of the temperature of CPZ-treated roots can explain the discrepancy in the rates of the oxygen consumption and heat production. However, we do not exclude that some amount of heat can be released during the thermoequilibration of a sample, immediately after addition of CPZ to roots in the beginning of experiment and before the start of record (see Section 2).

In conclusion, it seems that the early response of cells to the CPZ and Val, membrane-active compounds with different mechanisms of action, was nonspecific changes in the conductivity of the plasma membrane of the root cells, as seen by the K^+ permeability and MP data. Prolonged exposure of roots to Val resulted in the recovery of the ion homeostasis of cells. This suggests that there are active adaptive reactions, the energy cost of which is associated with the recovery of the ion homeostasis of cells and signified by the increase in the rate of heat production (see Fig. 3). On the other hand, the more considerable disturbance of the ion homeostasis due to the effect of CPZ on the lipid component of the membrane probably causes an involvement of a wider spectrum of systems and energy cost in the response reactions and the recovery processes of the root cells.

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