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Heat production and respiration of wheat roots under the modulation of plasma membrane ion conductivity☆

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Abstract

The sensitivity of cells to stress factors is associated with the function of electrogenic pumps and depends on their energy conditions. Regulation of cell metabolism and corresponding energy expense can be achieved by means of shifts in ion homeostasis via changes in the structural and functional properties of the plasma membrane. In the present work, we studied the changes in the rates of respiration and heat production along with the electrical membrane potential (MP) in cells of the excised roots of 5 day old wheat seedlings (*Triticum aestivum* L.) while the ion conductivity of their plasma membranes was modulated by 20 μM valinomycin (Val) and 100 μM chlorpromazine (CPZ). It is shown that both of these compounds induced an enhancement of potassium ion loss. CPZ also reduces both respiration intensity and heat production after 2 h of exposure. The enhancement of K^+ loss caused by the specific K^+ ionophore, Val, had no disorganizing effect on plasma membranes, and was compensated by an increase in ATPase activity on the plasma membrane. The essential rise of plasma membrane ion conductivity induced by CPZ, the compound with a pronounced membrane effect, was followed by a considerable inhibition of root respiration and heat production. These results point to the lack of cell energy resources to eliminate the damaging effect of CPZ.

Keywords: Heat production; Ion transport; Respiration; Root cells

1. Introduction

Cellular surface plays a significant role in the response of plant cells to stress factors. Structural and functional conditions of the plasma membrane determine both the early reactions and the processes related to adaptation. Change in the transport processes of the plasma membrane relates to early reactions of cells to stress factors. Most such actions cause a reduction in the electrochemical gradient of H^+ on the plasma membrane which starts the response reaction of cells. Regulation of the sensitivity of cells to injury is connected with activity of the electrogenic pump and depends on energy conditions. Power expenditures are closely connected with dynamics of the structure and function of cells and reflect the response of cells to external effects.

Respiration is a basic physiological process which generates energy and is responsive to changes in adaptive reactions. Heat liberation in non-photosynthesizing cells of plants occurs during the functioning of the respiratory chain of mitochondria and in oxidative reactions not connected with the mitochondrial apparatus. Heat production also requires power expenditures during ion transfer through the membranes (in particular, the plasma membrane).

Excised roots are used as the experimental model to investigate the reactivity and mechanisms of plant adaptation. There are few studies on the contribution of ionic transport to the heat production of plant cells [1]. In the present work we studied in excised wheat roots the influence of modulators of the plasma ionic conductivity on respiration, heat production, electrical membrane potential, potassium ion contents

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and pH. This allowed us to study special features of the regulation of energy processes following alteration of plasma membrane ionic conductivity.

2. Materials and methods

The objects of investigation were excised roots of 5 day old seedlings of spring wheat (*Triticum aestivum* L.). After overnight soaking in tap water, seeds were put on a glass covered with damp gauze and placed into a dish containing the cultivation solution. Seedlings were germinated in distilled water at room temperature. This technique makes it possible to avoid anaerobiosis and the roots do not exhibit a deficiency of moisture and air. Furthermore, during the experiment it is easy to extract seedlings with the intact roots from the gauze without damage and ruptures. The roots (2–3 mm from caryopsis) were cut from the seedlings and immediately used for experiments.

The rate of respiration of the excised roots was determined by measuring oxygen consumption and CO_2 release. Oxygen consumption and CO_2 release were measured by the Warburg manometric method. The excised roots (≈150 mg) were placed into the manometric vessels (20 ml) containing 3 ml of control or experimental solution and shaken with 110 rpm at 30 °C. After stabilization of temperature (10–15 min), the readings were taken every 60 min during 2 h. O_2 consumption and CO_2 release were registered simultaneously in each experiment.

Data are presented in μl of O_2 or CO_2 per hour per gram of fresh weight.

Heat production was measured by differential microcalorimeter (LKB-2277 Bio Activity Monitor, Sweden) which has a modularized circuit.

The content of potassium ions released by roots into incubation solutions was determined using the Phlapho-41 (Germany) flame photometer. Control of incubation medium pH was carried out using a pH-meter (Radelkis OP-21/1, Hungary). For all measurements 150 mg of excised roots were incubated in different media (3 ml) with moderate shaking (110 rpm) for 2 h at 30 °C.

The electrical membrane potential (MP) of root cells was measured using standard microelectrode techniques [2]. Glass microelectrodes with the aid of micromanipulator were introduced into the cells of the rhizodermis in the root elongation zone (1.5–2 cm from the root tip). The microelectrode and reference electrode were connected to the amplifier by AgCl electrodes via agar–agar bridges. The experiments were performed in 3–5 analytical and 3–7 biological replicates.

The data in Tables 1–3 present the mean and standard error values. Table 4 presents the results from one of three representative experiments.

In the present work compounds were used having different degrees of action on the structure and functional properties of plasma membrane. These were the specific potassium ionophore – valinomycin (Val, 20 μM) and chlorpromazine (CPZ). The concentration of Val used was selected from previous experiments [3].

CPZ is a membrane active compound with a wide spectrum of action. It is used as an inhibitor of Ca^{2+}/calmodulin dependent processes, membrane ATPases and phospholipases [4–6]. CPZ binds with polar groups of phospholipids [7]. Being a competitor with Ca^{2+} for binding in the membrane, CPZ displaces Ca^{2+} from the plasma membrane [4]. In the present work we used CPZ in a concentration of 100 μM,

Table 1
Effect of Val and CPZ on the membrane potential of excised roots cells

	Time of root incubation (h)	MP (mV)
Control (H_2O)	1	73.1 ± 0.6
	2	74.6 ± 0.4
Val (20 μM)	1	65.9 ± 0.8
	2	65.7 ± 0.5
CPZ (100 μM)	1	45.0 ± 0.3
	2	40.9 ± 0.2

Figures are given ± SD, n = 5.

Table 2
Effect of Val and CPZ on the K^+ efflux from roots and the pH of incubation medium

	Time of root incubation (h)	K^+ efflux from roots (μeq/g f.w.)	pH of incubation medium (rel. un.)
Control (H_2O)	1	2.08 ± 0.17	
	2	1.01 ± 0.10	5.95
Val (20 μM)	1	5.65 ± 0.31	
	2	5.80 ± 0.23	6.05
CPZ (100 μM)	1	9.58 ± 0.27	
	2	14.70 ± 0.38	6.40

Figures are given ± SD, n = 5.

Table 3
Effect of Val and CPZ on the respiratory gas exchange of the excised roots cells

	Time of root incubation (h)	O_2 consumption (μl/g f.w.)	CO_2 release (μl/g f.w.)	RQ
Control (H_2O)	1	624 ± 21	643 ± 25	1.03
	2	611 ± 26	593 ± 17	0.97
Val (20 μM)	1	635 ± 13	629 ± 21	0.99
	2	595 ± 17	589 ± 18	0.99
CPZ (100 μM)	1	522 ± 18	647 ± 19	1.24
	2	423 ± 23	404 ± 22	0.96

Figures are given ± SD, n = 3–5.

Table 4
Heat production by excised roots treated with Val and CPZ

	Time of root incubation (h)	Heat production (J/g f.w.)
Control (H_2O)	1	13.65
	2	13.40
Val (20 μM)	1	12.97
	2	13.30
CPZ (100 μM)	1	8.95
	2	9.75

Data present the results from one of 3 representative experiments.

which had been selected for excised roots in previous experiments [8].

3. Results

CPZ caused a significant increase in the K^+ efflux from the roots and a decrease of the cell MP value which enhanced with time (Tables 1 and 2). Furthermore, an increase of the pH of the root medium after 2 h incubation in the presence of CPZ was observed as compared with the control (H_2O) (Table 2). An increase of pH indicates that protons enter the cells of the roots. The lowering of MP (about 30 mV) is caused by the dissipation of the proton gradient on the plasma membrane. The value of a MP is determined by the relationship of plasma membrane electrogenic channels and channels of leakage. Reducing the MP of root cells in the presence of CPZ correlated with increased loss of potassium ions by excised roots is connected with the increase of the quantity of leakage channels. The lowering of the MP of the cells, the increased loss of the potassium ions, and the pH value of the incubation medium, point to the essential disturbance of ionic gradients and an increase in the ionic conductivity of the plasma membrane under the influence of CPZ.

The changes in respiratory gas exchange of roots in the presence of CPZ are connected with the violation of cellular ion homeostasis. CPZ caused a decrease in O_2 consumption and ambiguous alterations of the RQ (Table 3). The loss of potassium by cells in the presence of CPZ and the entrance of protons into the cytoplasm influences its pH, which is reduced in parallel to the reduction of the concentration of potassium in the cytosol [9]. It was shown [10] that during reduction in the concentration of potassium and the pH of the cytoplasm, the suppression of respiration was observed, caused by the action of cations on the redox reactions of the respiratory chain. After 1 h of CPZ action, O_2 consumption by the excised roots was reduced. CO_2 release remained at the control level, and RQ increased. The increase of the RQ after 1 h of root incubation in the solution with CPZ probably is connected with the inhibition of the respiratory chain of mitochondria, while the previous donors of electrons (in glycolysis) continue their normal function. After 2 h of root incubation in the solution with CPZ, the RQ value was close to that of the control. This is caused, apparently, by intensification of the membrane effect of CPZ with time and by unspecific inhibition of the respiration enzyme systems including glycolysis.

Respiration compensates for the loss of intracellular energy and supports the energy level necessary for fulfilling vital functions. Part of the energy released during respiration is one of the sources of heat production. Along with significant suppression of root respiration, diminished heat production was observed under the influence of CPZ (Table 4).

The K^+-ionophore Val caused a small loss of potassium ions by excised roots and lowered cellular membrane potential as compared with the control (Tables 1 and 2). These changes were less significant than in the presence of CPZ and did not enhance with time. The pH value of the root medium incubated with Val varied insignificantly (Table 2). The increase in K^+ conductivity of the plasma membrane of cells after a 2 h incubation of roots in the presence of Val had little influence on respiratory gas exchange and heat production (Tables 3 and 4). The RQ was not changed (Table 3).

4. Discussion

Restoration processes directed toward preventing destructive changes in ionic homeostasis are connected with the activation of a proton pump, in particular, H^+-ATPase of the plasma membrane, which uses metabolic energy for generation of the MP and transfer of K^+ through the membrane [11]. One of the mechanisms for plasma membrane H^+-ATPase regulation under stress conditions is the modulation of its activity by potassium ions both from internal and external sides of the membrane [12]. Zheng et al. [13] showed the stimulating influence of K^+ efflux into the incubation medium on the activity of H^+-ATPase. The proton ATPase of the plasma membrane is activated when the extracellular K^+ ion concentration achieves a definite level. Previously we showed that increased loss of the potassium ions by the cells of the excised roots in the presence of K^+-ionophore Val was compensated by small increase in ATPase activity on plasma membrane, as revealed by cytochemical methods [14].

The strong effect of CPZ on the studied parameters (significant increase in the K loss by cells, alteration of pH of the incubation medium, lowering of cellular MP, inhibition of root respiration, and heat production) points to the nonspecificity of the CPZ action. The marked enhancement of passive ion transport and considerable violation of the ion homeostasis (including Ca^{2+}) under the influence of CPZ is not compensated by the mechanisms of ion homeostasis maintenance localized on the plasma membrane (H^+-ATPase and Ca^{2+}-ATPase), since CPZ inhibits membrane ATPases.

At the early stages of the cellular response reaction under the effect of compounds which are distinguished by their membrane effect, the unspecific changes in the conductivity of the plasma membrane for K^+ and heat production of the excised roots occurs. In the presence of the specific K^+ ionophore Val, restoration of the ionic homeostasis of cells occurs with time and is accompanied by energy expenditures. There is a significant increase in potassium loss by roots in the presence of CPZ, caused by its effect on the structure of the plasma membrane and its functional components, connected with the membrane transport (ATPases). This is accompanied by a reduction in the intensity of respiration and heat release. The extent of this latter depends on the membrane effect of CPZ and its influence on cytoplasmic pH, the value of which is the basic regulator of the cell's homeostasis. The significant suppression of root respiration due to CPZ action, which is enhanced with time, indicates that the energy resources of cells are not sufficient for eliminating its damaging effect.

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