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Heat production of root cells upon the dissipation of ion gradients on plasma membrane[1]

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Abstract

The dissipation of ion gradients across plasma membranes contributes to the heat production by live cells. The aim of the present research is to determine the dependence of the rate of heat production by plant tissues on ion balance shifts, supposing a priori that the shifts are the beginning mechanism in adaptation to various stresses. Excised wheat roots subjected to prolonged incubation in different solutions served as objects for investigation. Two ion transporters (K^+/H^+-transporter nigericin and Ca^{2+}–ionophore A23187) shifted the ion homeostasis and induced distinct changes in the energy metabolism of plant tissues. Nigericin, which considerably increased the plasmalemma conductivity for protons and potassium, enhanced production of heat by root cells throughout the exposure. Prolonged incubation with A23187 was required to display its ion-transporting properties which were accompanied by a rise in the rate of heat release. The high rate of heat release and respiration in root cells exposed to ion transporters is a reflection of the ion-gradient dissipation and the increased energy expenditure for the activation of ATPase systems necessary for the restoration of ionophore-disturbed ion homeostasis. © 1998 Elsevier Science B.V.

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

1. Introduction

According to present views, some heat sources are activated during oxidative reactions, and others during hydrolysis of ATP [1]. The heat production thus accompanies each equalization of chemical potentials in a cell. Dissipation of ion gradients is supposed to greatly contribute to heat production. Ion gradients on cell membrane have been shown to serve as a reserve of energy, in the membrane, which may be used for cell activity [2].

The main goal of our work was to study the energy exchange in cells of whole plant tissue under the action of specific ionophores, transporting ions via the plasma membrane according to the concentration gradient. To obtain a comprehensive picture, not only the rate of released heat but the rate of oxygen consumption and changes in the ion contents of cells had to be determined.

2. Experimental

The excised roots of 5-day old wheat seedlings grown in $CaCl_2$ (0.25 mM) were used as the subject of investigation.

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Heat production by root cells was measured by a differential microcalorimeter (LKB-2277 Bio Activity Monitor, Sweden). Glass vials with a total volume of 3 cm^3 were used in our experiments.

Oxygen consumption was measured by the Warburg manometric method.

K^+ content of the incubation solution was determined by the Phlapho-41 (Germany) flame photometer.

Ca^{2+} content of roots was measured by means of AAS-IN (Germany).

Changes in proton content of the incubation solution were measured by pH-meter.

Ion shifts in root cells were reached by the addition of ionophores to the incubation solution: nigericin 0.01 mM (electroneutral K^+/H^+ transporter) and antibiotic A23187 transporting Ca^{2+} and H^+.

3. Results

Root incubation with nigericin increased the heat production rate (Fig. 1). For the first 3 h, we assume that the increase is connected with the dissipation of K^+ and H^+ gradients on plasmalemma. Indeed, K^+ efflux occurring in response to the cutting off roots and putting them to the incubation solution was considerably larger under the action of nigericin (Fig. 2). At the same time, there was H^+ influx into cells (Fig. 3). On average, the pH level of the incubation solution was raised by 0.7 unit.

There was contrary action of nigericin on heat production and oxygen consumption rates during the first hours of incubation. Heat production was enhanced, but respiration decreased (Fig. 4).

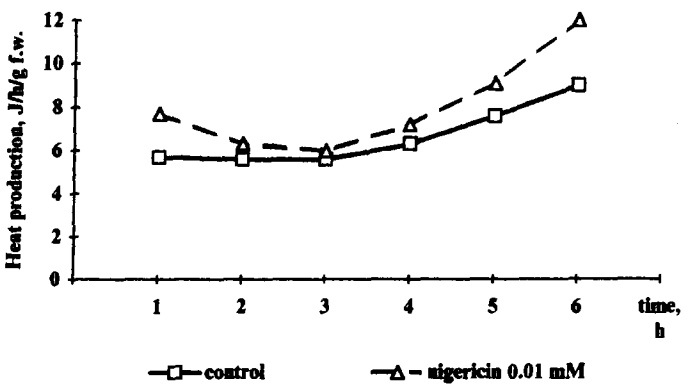

Fig. 1. Heat production by roots cells under the action of nigericin (0.01 mM).

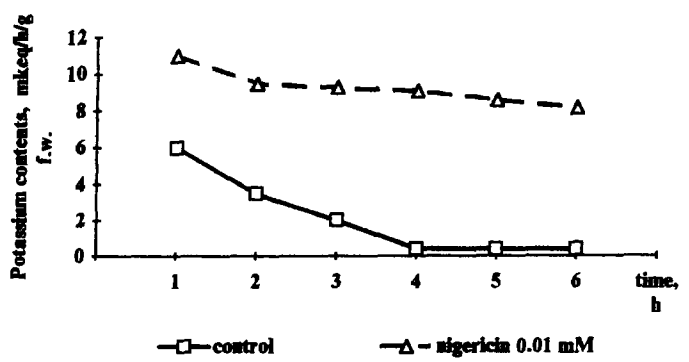

Fig. 2. Effect of 0.01 mM nigericin on the K^+ efflux from excised roots.

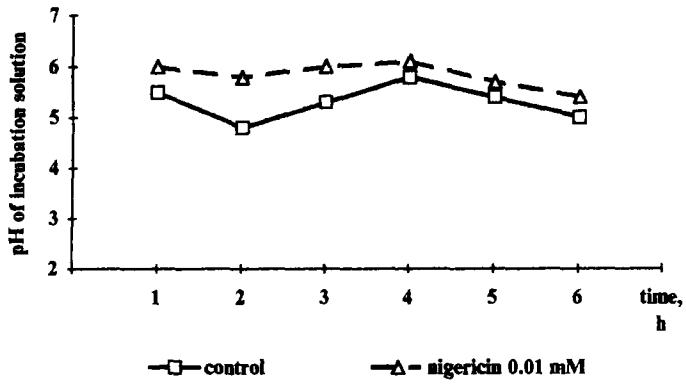

Fig. 3. Effect of 0.01 mM nigericin on the pH of the incubation solution of excised roots.

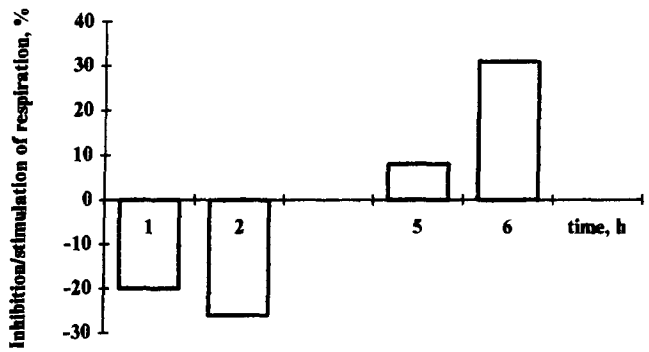

Fig. 4. Respiration of root cells under the action of nigericin 0.01 mM (inhibition/stimulation×% compared with control).

Prolonged exposure to nigericin was accompanied by an increased rate of heat production (Fig. 1) as well as increased O_2 consumption rate (Fig. 4). These effects are probably connected with increased energy expenditure for active ion transport. The data of Fig. 2 demonstrate that although K^+ ions previously emanating from cells of control version were fully consumed by the end of incubation, it did not occur under the action of nigericin.

Ca–ionophore A23187 did not change the K^+ and H^+ fluxes (Table 1yy) or Ca^{2+} transport (Table 1) during the first hour of exposure. At the same time, there

Table 1
Effect of Ca-ionophore A23187 (0.01 mM) on the pH of incubation solution (K^+ efflux from roots and Ca^{2+} content of roots during 1-h and 6-h exposures)

	Time of root incubation/h	pH of incubation solution/(rel.un.)	K^+ efflux from roots/(μeq/h/g f.w.)	Ca^{2+} content of roots/(μeq/h/g f.w.)
$CaCl_2$ (0.25 mM)	1	5.3±0.14	3.2±0.02	2.39±0.17
	6	6.0±0.00	3.42±0.14	3.42±0.14
$CaCl_2$ (0.25 mM)	1	5.6±0.10	3.6±0.01	1.55±0.05
-A23187 (0.01 mM)	6	6.4±0.00	2.7±0.05	4.08±0.02

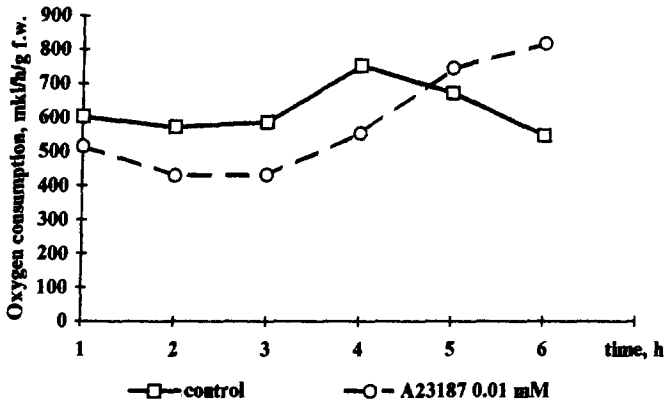

Fig. 5. Respiration of roots cells under 0.01 mM Ca–ionophore A2387 exposure.

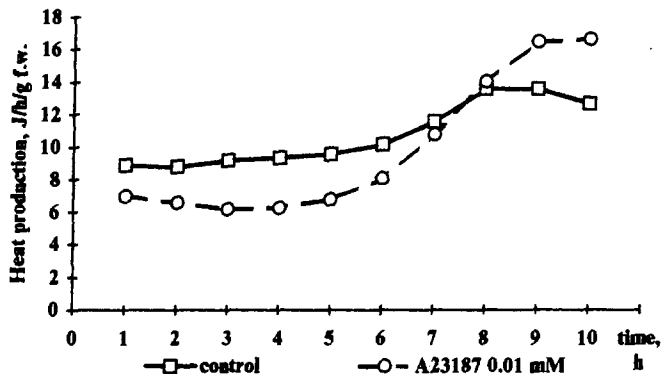

Fig. 6. Heat production by root cells under 0.01 mM Ca–ionophore A23187 exposure.

was a significant depression of the rates of oxygen consumption (Fig. 5) and heat production (Fig. 6).

Prolonged exposure to A23187 was accompanied by the same changes in the K^+ and H^+ fluxes with nigericin (Table 1) as by strengthening of Ca^{2+} influx into cells (Table 1). The effects of prolonged exposure, apparently, demanded an additional energy expenditure for restoration of ion homeostasis which led to an increase of respiration (Fig. 5) and heat production (Fig. 6).

4. Discussion

Disturbance of ion homeostasis is one of the primary cell responses to stress and starts adaptation in live cells and tissues [3]. Hence, artificial dissipation of ion gradients on plasma membranes may be followed by adaptive reactions and development.

The two ion transporters used in our experiments shifted ion homeostasis to different extents and induced some distinct changes in energy metabolism of plant tissues. Nigerecin caused significant gradient dissipation of potassium ions and protons which are usually considered as the main potential-determining and energy-storing ions (Figs. 2 and 3). Additional energy expenses were expressed as increased heat production during nigericin exposure (Fig. 1). Antibiotic A23187 did not cause considerable shifts of ion homeostasis during the first hour of exposure, but there was marked dissipation of ion gradient only with long time incubation (Table 1). The

time course of these changes explains the curve of heat production under the action of A23187 (Fig. 6). Only great changes in ion homeostasis are accompanied by enhancement of the heat release.

The respiration rate upon both nigericin and A23187 action was depressed during the first hours and was stimulated by the end of incubation. One of the reasons for the respiration depression upon the action of nigericin and A23187, probably, is cytoplasm acidification by proton transport in cells. According to our previous data, A23187 initially acts as a slight protonophore [4]. Nigericin has also been shown to decrease the respiration of bacterial cells due to cytoplasm acidification [5]. It has even been pointed out that nigericin might be used as an agent for decreasing the pH of cytoplasm, thereby hampering DNA synthesis [6].

Ionophores increasing membrane conductivity for K^+ and H^+ may cause failure of adaptive processes owing to excessive energy expenditure for active ion transport and restoration of ion homeostasis. It is quite possible that a realization of adaptation processes is hampered by inhibition of DNA synthesis due to cytoplasm acidification as well.

5. Conclusions

Heat production by plant cells is connected with the dissipation of ion gradients, in particular of potassium ions and protons. Prolonged effects of nigericin and A23187 induce an increase in the rate of heat production, which reflects not only the dissipation of ion gradients but the energy expenditure for active ion transport tending *toward* restoration of disturbed ion homeostasis.

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