Some aspects of the phytotoxic action of fusaric acid on primary *Ricinus* roots

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ABSTRACT

Fusaric acid, at a concentration of 1mM induced at pH 5.5 an early hyperpolarisation was followed by a marked depolarisation of membrane potential difference. During this time increased electrolyte leakage from the primary *Ricinus* roots was determined. At higher pH values (6.5 and 8) the depolarisation caused by fusaric acid was immediate without hyperpolarisation observed at pH 5.5. Simultaneous exposure of the roots to P-ATPase activator fusicoccin and fusaric acid (pH 6.5) diminished the hyperpolarising effect of fusicoccin. The present results suggest that the dissociated form of fusaric acid does directly affect particular cell targets (plasmalemma, mitochondria) and viability of root cells decreased with the time of exposure and concentration of fusaric acid.

Keywords: fusaric acid; membrane potential; electrolyte leakage; *Ricinus* roots

Fusaric acid is a toxin produced by fungi genera *Fusarium* and *Giberella,* causing wilt disease of a great variety of plants (Gäumann 1957). It alters the permeability of plant membranes (Gäumann 1958, Pavlovkin 1998), increases electrolyte leakage (Linskens 1955, Arias 1985, Marrè et al. 1993), causes modification of membrane potential (Köhler and Bentrup 1983, D'Alton and Etherton 1984, Marrè et al. 1993, Pavlovkin 1998), inhibits respiratory activity (Kuo and Scheffer 1964, Arias 1985, Marrè et al. 1993) and decreases ATP levels (Köhler and Bentrup 1983, D'Alton and Etherton 1984) in several plant species. However, fusaric acid was one of the first fungal metabolites implicated in the pathogenesis. Its mechanisms of action are still unclear.

The aim of the present electrophysiological experiments was to characterise the fusaric acid-induced changes of membrane potential of intact *Ricinus* primary roots. P-ATPase stimulator fusicoccin was used to examine the effect of fusaric acid on the P-ATPase in intact roots.

MATERIAL AND METHODS

Plant material. *Ricinus communis* L. var. *gibsonii*, cv. Carmencita (Walz Samen, Stuttgart, Germany was grown in standard potting soil under universal white fluorescent lamps, irradiance of 200 μmol/m^2/s (L 58 W/25, Osram, München, Germany) in growth chambers. They were exposed to 14 h light and 10 h darkness among 21°C with 60–80% relative humidity. Twenty-one day old plants were used for electrophysiological measurements.

Electrophysiological measurements. The primary roots were mounted in a vertical Plexiglas chamber that was perfused with 1X solution (containing 1mM KCl, 1mM $Ca(NO_3)_2$, 1mM NaH_2PO_4, 0.5mM $MgSO_4$, pH 6.5) in which fusaric acid (final concentration 0.5, 1 or 2mM) was dissolved. Acidification by the addition of fusaric acid to nutrients solutions was neutralised by NaOH. For pH study at pH 5.3 was pH of control medium adjusted by 1mM MES. Diffusion potential was determined by the addition of 1mM NaCN + 1mM salicylhydroxamic acid (SHAM) to the perfusion solution. To test whether the P-ATPase activity of root cells could be differently enhanced either directly at the membrane level, or indirectly by energy supply, the P-ATPase stimulating toxin fusicoccin (in 0.1% ethanol, final concentration 15μM) was added to the perfusion solution. Measurements of the membrane potential were carried out at 22°C, by use of the standard microelectrode techniques which have been earlier described in detail (Marx and Ullrich-Eberius 1988). The microelectrode inserted into the outer cortex cells 10 mm from the root tip. Insertion of the microelectrode was observed under the microscope (× 120). Each experiment was repeated at least three times.

Electrolyte leakage. The primary roots of 21 days old *Ricinus* plants were incubated in 0.5mM $CaSO_4$, 1mM MES, with/without fusaric acid (0.5, 1 or 2mM), pH 5.5. The overall amount of electrolytes released by the root cells over the time was determined by monitoring the changes of the specific conductivity of the incubation medium. A conductivity meter (OK-109-1, both manufactured in

Supported by the Slovak Grant Agency VEGA, Project No. 3/23051/23.

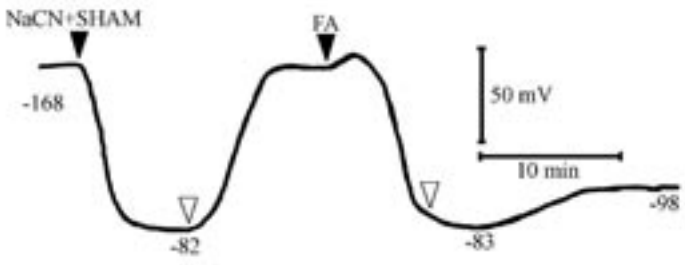

Figure 1. Representative changes of membrane potential of *Ricinus* outer cortical root cells after 1mM NaCN + 1mM SHAM treatment and followed by depolarisation of membrane potential by 1mM fusaric acid (FA) addition (pH 5.5); numbers at the traces denote recorded millivoltage (filled triangle – addition, empty triangle – removal of tested substances)

Hungary) equipped with a bell-shaped measuring electrode (OK-902) was used for this purpose.

RESULTS

The primary *Ricinus* roots are suitable objects for electrophysiological measurements (Mistrik et al. 2000). When the standard 1X nutrient solution was used in the perfusion chamber the value of membrane potential of the primary *Ricinus* root cortex cells were about –100 mV and the first two hours gradually hyperpolarised up to –153 to –179 mV (–169 ± 14, $n = 62$). Thereafter, membrane potential was constant, at least, for one day.

Two components of membrane potential were separated by an inhibitor of oxidative phosphorylation 1mM (NaCN + SHAM). The addition of inhibitors to 1X solution depolarised membrane potential to the level of diffusion potential (Figure 1). Fusaric acid (1mM) added after recovery of the membrane potential depolarised the membrane potential on the same level as NaCN + SHAM.

The concentration effect was performed to see if the effects of fusaric acid on membrane potential changed gradually with fusaric acid concentration. The magnitude of the depolarisation increased with the fusaric acid concentration (Figure 2). The concentration of fusaric acid considerably affected the depolarisation rate. Higher concentrations of fusaric acid caused depolarisation that was faster than those caused by lower concentrations (tracing are not shown), but were only slightly greater in magnitude. In all measurements partial re-polarisation of membrane potential was registered when fusaric acid was being removed and the treatment by fusaric acid did not exceed 10 min.

The results presented in Figure 3 show that the magnitude of depolarisation increased with the exposure time of 1mM fusaric acid. Exposures from 4 to 10 min depolarised the membrane but after removing of fusaric acid membrane potential was particularly re-polarised. The exposures exceeding 10 min depolarised membrane potential to the level of lower diffusion potential and after the removal of fusaric acid membrane potential was newly re-polarised.

We tested the effects of different pH levels on the membrane potential changes caused by 1mM fusaric acid (Figure 4a–c). At pH 5.3 there

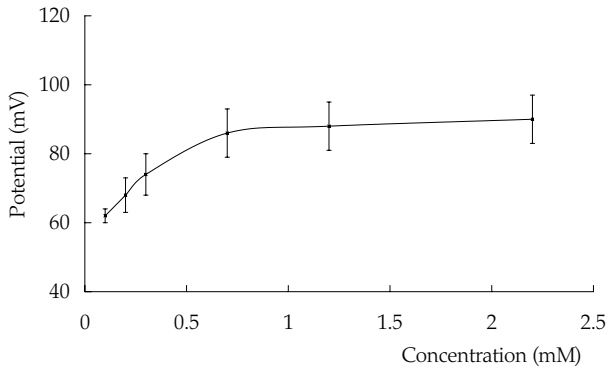

Figure 2. Effect of different concentrations of fusaric acid at membrane potential of *Ricinus* primary root cells; the values are mean ± *SD*, $n = 3$

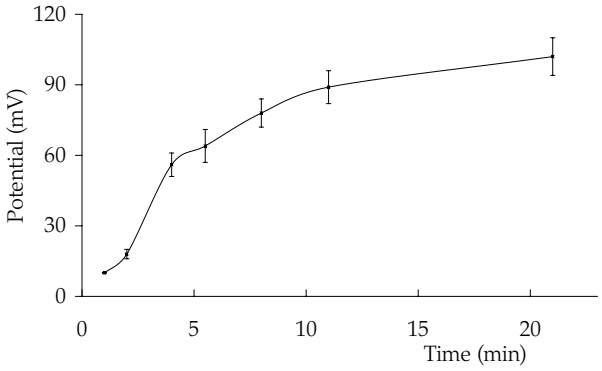

Figure 3. Effect of 1mM fusaric acid, pH 6.5, at different exposure time on membrane potential of *Ricinus* primary root cells; the values are mean ± *SD*, $n = 3$

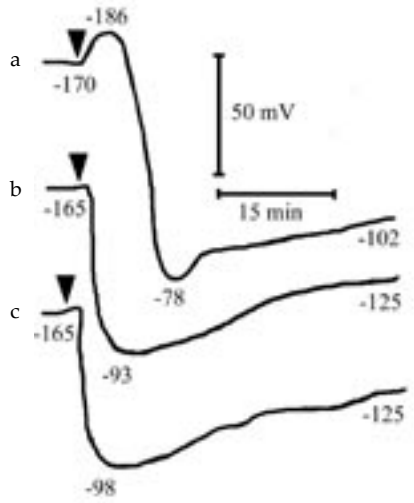

Figure 4. Tracings of chart recordings showing the effects of 1mM fusaric acid at three different pH levels (A – pH 5.3, B – pH 6.5, C – pH 8); numbers at the traces denote recorded millivoltage; exposure time of fusaric acid was 4 min (filled triangle – addition of fusaric acid)

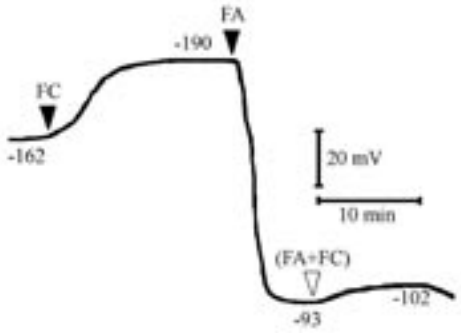

Figure 5. Representative membrane potential measurements of outer cortex cells; effect of 15μM fusicoccin (FC) on membrane hyperpolarisation and followed depolarisation of membrane by 1mM fusaric acid (FA), pH 6.5, exposure time of fusaric acid was 8 min

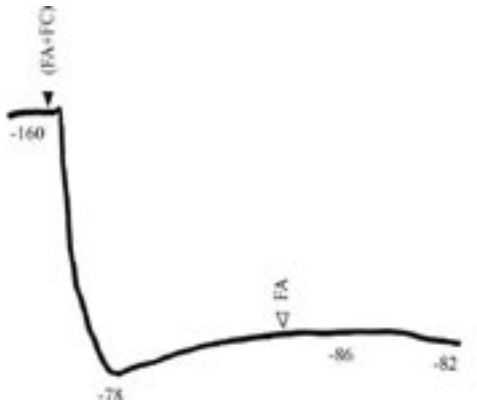

Figure 6. Simultaneous application of 1mM fusaric acid (FA) + 15μM fusicoccin (FC), and typical fusaric acid depolarisation (pH 6.5); exposure time of fusaric acid was 20 min

was an initial hyperpolarisation upon addition of fusaric acid (Figure 4a) which was followed by evident membrane potential depolarisation. After reaching a depolarisation maximum, membrane potential was partially re-polarised. The magnitude of membrane potential hyperpolarisation was about –20 mV. At pH 6.5 (Figure 4b) and 8.0 (Figure 4c) the initial effect of fusaric acid was an immediate depolarisation, which was followed by partial membrane potential repolarisation.

Fusicoccin, a non-host-specific fungal toxin, hypepopolarise cell membranes of higher plants, probably due to the stimulation of the P-ATPase, an electrogenic H^+ pump (Marrè 1979). In the present study, fusicoccin was used to test the state of the electromotive force regulating plasmalemma H^+-ATPase during the course of fusaric acid treatment. Fusicoccin rapidly and permanently hyperpolarised the membranes of the outer cortex *Ricinus* cells. The membrane potential values reached approximately –30 mV (Figure 5) in the control cells. When fusaric acid was applied after pre-treatment of the roots with fusicoccin, the membrane depolarisation by fusaric acid in comparison to the control considerably decreased by 12 ± 2 mV (n = 3) (Figure 5).

Moreover, simultaneous addition of fusicoccin and fusaric acid in the perfusion solution completely prevented the hyperpolarisation effect of fusicoccin and 20 min influence of fusaric acid caused depolarisation membrane potential (Figure 6).

The conductivity of the incubation medium of *Ricinus* enhanced by increasing concentrations of fusaric acid (Figure 7). A larger increase in electrolyte leakage was observed et 2mM fusaric acid, indicating, that above a certain threshold the concentrated fusaric acid caused severe membrane damage.

DISCUSSION

Fusaric acid is known for its interaction with a number of extra- and intracellular structures (Marrè et al. 1993). Therefore many different mechanisms of fusaric acid phytotoxicity have been suggested (Köhler and Bentrup 1983, D'Alton and Etherton 1984, Marrè et al. 1993, Pavlovkin 1998). In general, these mechanisms include disruption of the plasma membrane transport processes, which can result in perturbation of plant nutrition and metabolism. *Ricinus* roots appear to provide a suitable material for studying mechanisms in action of this toxin on ion transport (Mistrik et al. 2000). Here measured responses of electrical membrane potentials changes of primary *Ricinus* root cells to fusaric acid are very similar to those previously described for other plant tissues such as tomato

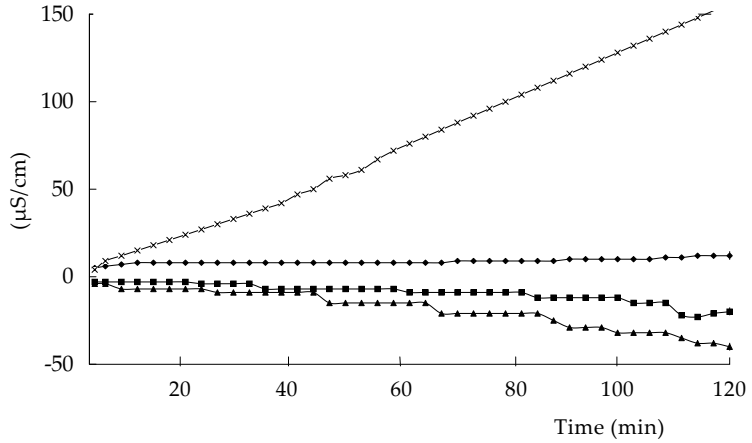

Figure 7. Time course of electrolyte leakage of *Ricinus* primary root segments after treatment with different fusaric acid concentrations (▲ control, ■ 0.5mM, ● 1mM, × 2mM fusaric acid), pH 5.5

roots (D'Alton and Etherton 1984), maize roots (Pavlovkin 1998) and *Egeria densa* leaves (Marrè et al. 1993).

The fact, that fusaric acid induces an early hyperpolarisation followed by a marked depolarisation of membrane potential in *Ricinus* roots is very similar to results reported for tomato roots (D'Alton and Etherton 1984), corn roots (Pavlovkin 1998) and *Egeria densa* leaves (Marrè et al. 1993). D'Alton and Etherton (1984) proposed that the initial hyperpolarisation could be due to an early stimulation of the proton pump by the acidification of the cytosol consequent on the entry in to the cell of the undissociated form of the fusaric acid. Such hypothesis is supported by the findings of Marrè et al. (1993), in which fusaric acid induces acidification of cell sap in *Egeria* leaf cells. On the other hand, the depolarisation observed after about 5 min of treatment with fusaric acid could be due either to direct inhibition of oxidative phosphorylation, and, through an energy shortage, to the inhibition of the P-ATPase pump, or to a general toxic effect on intracellular structures.

In *Ricinus* roots (results not showed), as previously reported for other plant materials (Linskens 1955, Gäumann 1958, Dunckle and Wolpert 1981, Marrè et al. 1993, Pavlovkin 1998), fusaric acid induced a marked increase of electrolyte leakage, which can represent particular manifestations of more general effects on the membranes.

To characterise the immediate effect of fusaric acid on the P-ATPase of root cells a set of experiments with fusicoccin. The results showed that the functional activity of P-ATPase is directly influenced by fusaric acid, because fusaric acid directly influenced fusicoccin caused hyperpolarisation of membrane potential. This response may point to dependent sites or modes of action of an alternation of the P-ATPase activity by fusaric acid and fusicoccin. With prolonged exposure to fusaric acid, the membrane continued to depolarise and this depolarisation became irreversible. One possible explanation, for this irreversible depolarisation is, that fusaric acid cause reduction in ATP levels (Köhler and Bentrup 1983, D'Alton and Etherton 1984) that are necessary for electrogenic extrusion of H^+ and hence maintenance of the membrane potential. Other investigators have found that fusaric acid reduces respiratory rates in tomato and *Egeria* plants (Naeff-Rooth and Reusser 1954, Marrè et al. 1993).

This electrophysiological approach to the study of possible membrane-mediated mechanisms of fusaric acid toxicity indicates that fusaric acid has direct and indirect effects on the plasma membrane. The direct effect was indicated by a rapid depolarisation of membrane potential. The indirect effects are indicated by a transient hyperpolarisation followed a slow depolarisation, possibly caused by reduced ATP levels inhibiting electrogenic extrusion and that can involve specific interactions with particular cell targets.

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Received on December 2, 2003

ABSTRAKT

Některé aspekty fytotoxického působení kyseliny fuzariové na primární kořeny skočce

Kyselina fuzariová v koncentraci 1mM (pH 5.5) indukuje hyperpolarizaci membránového potenciálu, po které bezprostředně následuje jeho depolarizace a zvýšené vytékání elektrolytů z kořenů rostlin skočce. Při zvýšeném pH (6.5 a 8.0) byla depolarizace pozorována bezprostředně po přidání kyseliny fuzariové do průtokového roztoku. Současné ošeření kořenů aktivátorem H^+-ATPázy fuzikokcinem a kyselinou fuzariovou (pH 6.5) potlačilo hyperpolarizační účinek indukovaný fuzikokcinem. Výsledky naznačují, že disociovaná forma kyseliny fuzariové má kromě přímého účinku na plazmatickou membránu kořenových buněk (depolarizace membránového potenciálu) také vliv nepřímý (mitochondrie). Rozsah poškození a reverzibilita vzniklých symptomů se zvyšovaly s délkou expozice a s koncentrací kyseliny fuzariové.

Klíčová slova: kyselina fuzariová; membránový potenciál; vytékání elektrolytů; primární kořeny skočce

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