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A CFTR chloride channel activator prevents $HrpN_{ea}$-induced cell death in *Arabidopsis thaliana* suspension cells

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Abstract

Erwinia amylovora is a necrogenic bacterium that causes fire blight of the Maloideae subfamily of *Roseacae*, such as apple and pear. It provokes necrosis in aerial parts of susceptible host plants and the typical hypersensitive reaction in non-host plants. The secreted harpin, $HrpN_{ea}$, is able by itself to induce an active cell death in non-host plants. Ion flux modulations were shown to be involved early in such processes but very few data are available on the plasma membrane ion channel activities responsible for the pathogen-induced ion fluxes. We show here that $HrpN_{ea}$ induces cell death in non-host *Arabidopsis thaliana* suspension cells. We further show that two cystic fibrosis transmembrane conductance regulator modulators, glibenclamide and bromotetramisole, can regulate anion channel activities and $HrpN_{ea}$-induced cell death.

Keywords: Anion channel; *Arabidopsis thaliana*; Cell death; CFTR; Harpin

1. Introduction

Erwinia amylovora is a bacterial pathogen that causes fire blight disease of apple, pear and other members of the *Rosaceae*, its host plants. It secretes the $HrpN_{ea}$ harpin, a "hypersensitive response" (HR) elicitor [42]. HR cell death is a response of non-host plant to pathogen attack and consists of a rapid necrosis at the site of infection that cordons off the pathogen and limits its spread [8,15]. There is a growing consensus that HR is similar to animal programmed cell death (PCD) and that ion channel regulation is a necessary event to induce PCD [15,16,21,31,44,45]. In plants, plasma membrane potential and ion flux variations are among the earliest signaling events detectable in response to pathogens and elicitors [30,32,34,45]. However, the underlying ion channel activities have been recorded rarely. Indeed, most of the electrophysiological studies on plant cells are performed with patch-clamp technique applied to protoplasts and it seems that the cell wall removing (protoplasts preparation) alters the cell response capacity to pathogen or elicitor [4,19]. Another approach to analysis of ion channels in intact cells that retains their cell wall is the microelectrode voltage-clamp technique. This technique allows long-term recording of the free running membrane potential and whole cell ion currents, the internal medium remaining physiological (composition non-controlled). We have shown previously, using single microelectrode voltage-clamp (DSEVC), that *Arabidopsis thaliana* suspension cells respond to the fungal elicitor hypaphorine in the same way as root hairs, its natural target [36]. Other studies demonstrated that suspension cultured cells are a powerful system of reduced complexity to analyze the signal transduction pathway induced by pathogens [7,29,43]. Thus, we used *A. thaliana* suspension cells to inves-

Abbreviations: AHAS, acetohydroxyacid synthase; AVD, apoptosis volume decrease; CFTR, cystic fibrosis transmembrane conductance regulator; DMSO, dimethyl sulfoxide; DSEVC, discontinuous single electrode voltage-clamp; HR, hypersensitive response; KORC, K^+ outward rectifying current; MAPK, mitogen activated protein kinase; PCD, programmed cell death; SU, sulfonylurea molecule.

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tigate early signaling events induced by $HrpN_{ea}$. We showed previously that $HrpN_{ea}$ could modulate K^+ and anion currents [10] suggesting indirect effects on the channel proteins. Regulation of anion fluxes was reported in pathogen-induced plant cell death. For example, a large nitrate efflux is necessary to induce cell death in tobacco in response to cryptogein [25,43]. Moreover, anion channel antagonists have been shown to interfere with elicitor or pathogen-induced responses such as Ca^{2+} influx [9], production of active oxygen species [17,35], MAPK activation [26], and phytoalexin synthesis [9,17]. In animal cells, Maeno et al. [27] showed that apoptosis volume decrease (AVD: cell shrinkage) is a major hallmark of PCD. This AVD is due to a strong activation of ion effluxes. In plant cells, the increase of anion effluxes in response to pathogen elicitors [43,45] is consistent with the AVD. However, in *A. thaliana* suspension cells, we observed a decrease in anion current (efflux decrease) in response to $HrpN_{ea}$ [10]. These data did not fit with the observations described above but are closely related to those reported for hepatoblastoma apoptosis [18]. In this last model, apoptosis is induced by the decrease in cystic fibrosis transmembrane conductance regulator (CFTR) anion currents (members of ABC transporter superfamily). In plant, CFTR modulators, were shown to be effective on slow anion channels of *Vicia faba* guard cells [23]. Moreover, Leonhardt et al. [24] showed, using antibodies, that slow anion channels are, or are closely, controlled by a polypeptide exhibiting an epitope shared with the mammalian CFTR. Lastly, AtMRP5, a protein of the ABC transporter superfamily, which has a high similarity to CFTR and which is sensitive to glibenclamide, was suggested to control ion channels [12]. In this study, our aim was to investigate the putative involvement of the anion current decrease in $HrpN_{ea}$-induced cell death on *A. thaliana* suspension cells (non-host plant) by using CFTR modulators, glibenclamide, a sulfonylurea (SU) molecule, as an inhibitor and bromotetramisole as an activator, according to the hypothesis developed on hepatoblastoma [18].

2. Results

$HrpN_{ea}$ at 5 μg ml^{-1} (0.13 μM), a classically used $HrpN_{ea}$ concentration [42,33], increases cell death in comparison with the cells treated with negative control (Table 1). According to our hypothesis we thus tested in our model the effect of glibenclamide and bromotetramisole. In animal systems, the concentrations of glibenclamide tested are highly variable: whole-cell CFTR-Cl^- currents are inhibited at a half-maximal concentration of 20 μM [39], apoptosis is induced at 1 mM [18]. Thus, we tested the effect of different concentrations of glibenclamide on anion currents. The deactivating currents (Fig. 1A), previously characterized as anion currents [3,10,36], are sensitive to glibenclamide in a dose-dependent manner (Fig. 1). The glibenclamide concentration for half-maximum inhibition of anion current observed in our model is about 7 μM (Fig. 1B), of the same order of magnitude as those observed for slow-type anion currents on plant cells [23]. Thus, we tested the effect of 10 μM glibenclamide on cell death. After 24 h, glibenclamide increased cell death, mimicking the $HrpN_{ea}$-induced cell death (Table 1). Although glibenclamide is effective on anion currents in our model (Fig. 1) and in *V. faba* guard cells [23,24], we checked if the glibenclamide-induced cell death might be due to other known glibenclamide or SU effects, i.e. K^+ channel inhibition [22] or herbicidal activity [5,6]. Glibenclamide (10 μM) does not induce a change in K^+ outward rectifying current (KORC) activity of *A. thaliana* suspension cells (ΔI_{KORC} at steady state for a + 80 mV voltage step = 1.3 ± 9%, $n = 6$, data not shown). Yet in plants, the herbicidal activity of SU family acts through AHAS inhibition [5,6]. AHAS catalyses the for-

Table 1
Modulation of $HrpN_{ea}$-induced cell death by anion channel modulators
Increase in cell death after a 24 h treatment with glibenclamide, $HrpN_{ea}$ alone or mixed with bromotetramisole. Variations are given as a percentage with respect to the control level. Data correspond to mean values ± S.D. and n is the number of independent experiments

	5 μg ml^{-1} $HrpN_{ea}$	10 μM Glibenclamide	5 μg ml^{-1} $HrpN_{ea}$ + 5 μM bromotetramisole
Cell death (%)	21 ± 3 ($n = 6$)	26 ± 4 ($n = 3$)	1.3 ± 2.8 ($n = 3$)

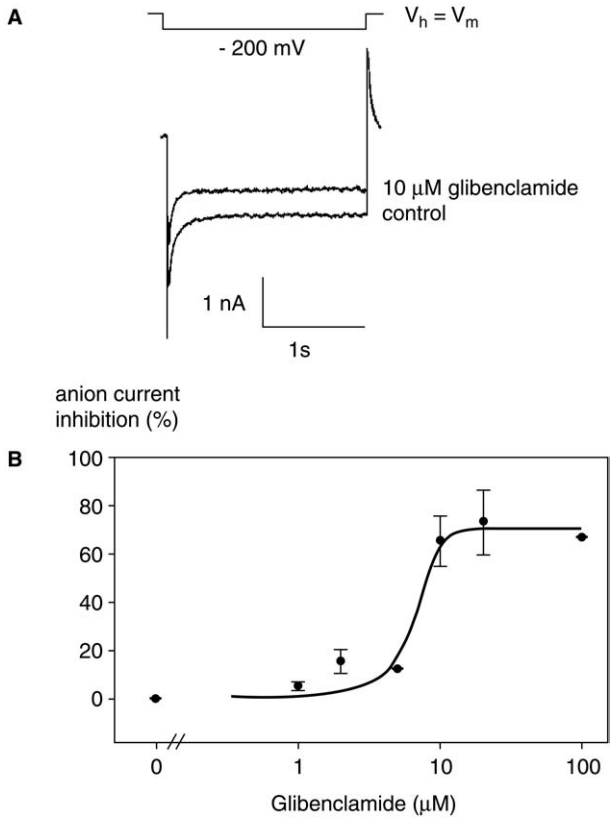

Fig. 1. Effect of glibenclamide on anion currents.
(A) Anion currents measured under control conditions and after adding 10 μM glibenclamide in the culture medium. Voltage pulses were –200 mV. Holding potential was V_m. (B) Dose-dependent decrease of anion currents as a function of glibenclamide concentrations. Data represent the maximal effect. They were obtained from at least three independent experiments and were fitted by a 5-parameter double exponential. The error bar corresponds to one standard error.