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Communication

Promotion of Stomatal Opening by Indoleacetic Acid and Ethrel in Epidermal Strips of *Vicia faba* L.

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ABSTRACT

Indole-3-acetic acid (IAA), at concentrations of 0.01 to 1.0 millimolar, and ethephon (0.3% v/v Ethrel) promote stomatal opening when applied to epidermal peels of *Vicia faba* L. in light or dark. The effect of ethylene is seen by 30 minutes and maximal opening (over two times that of untreated controls) occurs after only 60 to 90 minutes in the light. Stomatal opening by IAA and Ethrel in both light and dark is prevented by 0.14 millimolar AgCl. It is suggested that the effect of added IAA, but not that of light, is linked to ethylene production. The possible role of ethylene in stomatal opening during fungal infection is discussed. The stomates of *Vicia faba* provide a new system to study the effects of ethylene on certain membrane-regulated processes.

Stomates control gas exchange between the intercellular spaces of the leaf and the atmosphere, thereby regulating both transpiration and photosynthesis. It is well known that factors such as light, CO_2, humidity, temperature, K^+ concentration, and ABA alter stomatal pore size (25), but there are conflicting reports on the effects of the phytohormones IAA and ethylene.

IAA has no effect on the transpiration rates of barley (14) and oat leaves (15). There is also no effect of IAA (0.1 μM–0.1 mM) on stomatal opening of *Commelina communis* in CO_2-free air, but the hormone eliminates the closing response attributed to CO_2 (20). In contrast, Pemadasa (19) reports that IAA (10 μM–0.1 mM) causes stomata to open in *Commelina*, although Zelitch (26) found that stomata in tobacco close in response to the synthetic auxins 2,4-D and NAA.

With regard to ethylene, the gas has no effect on stomatal size of turgid leaves of *Zea* and *Pisum* in the light or dark (17) or of Pinto bean or *Sedum* leaves (16). Yet, ethephon, which is converted to ethylene by the cells, enhances stomatal opening in olive (22). But ethephon also closes stomata (8, 13) and ethylene gas decreases stomatal conductance in peanut leaves (18) and increases stomatal resistance in a number of plant species (23). Stomatal closure occurs also in leaves of tomato (7, 16) and carnation (16) after exposure to ethylene. Mudhaven *et al.* (16) note that at least 6 to 12 h is needed for this response.

In the present study, we report for the first time in *Vicia faba* that IAA and ethylene rapidly and markedly increase stomatal aperture in air under both light and dark conditions. Evidence is presented that the IAA effect is mediated by endogenously produced ethylene.

MATERIALS AND METHODS

Plants of *Vicia faba* L. were grown from seed (Carolina Biological Supply Co.) in a mixture of sterile soil, peat, vermiculite, and perlite (2:2:1:1) with limestone and fertilizer added. Plants were maintained in an environmental control chamber at a constant temperature of 25 ± 1°C with 100 μE m^{-2} s^{-1} of photon flux density during a 16-h photoperiod from time of sowing. Plants were watered daily to saturation to avoid water-stress and used when they were 3 to 4 weeks old.

The standard incubation medium consisted of 50 mM KCl and 10 mM Mes buffer adjusted to pH 6.15 with KOH. Ethephon (0.3% v/v Ethrel, a commercial preparation), AgCl (0.14 mM), or IAA singly or in combination were added as indicated. IAA was dissolved in ethanol. The maximum concentration of ethanol was 1% in 1 mM IAA: for these experiments controls also contained equivalent amounts of ethanol. All other compounds were dissolved in deionized water treated by ultrafiltration.

The epidermis from the abaxial side of the youngest fully expanded leaves was peeled at an acute angle to disrupt the epidermal cells and was trimmed into approximately 5 × 10 mm strips.

For treatments in the dark, epidermal peels were first floated in standard incubation medium in covered Petri dishes for 1 h in complete darkness at a constant temperature of 25 ± 1°C to close the stomates. The peels were then transferred to the test solutions under the same conditions. Dark manipulations and measurements were carried out under green light and a green filter was placed on the condenser of the microscope. For treatments in the light, the epidermis was peeled just prior to the start of the photoperiod. The peels were placed in covered Petri dishes in the incubation medium under a constant photon flux density of 100 μE m^{-2} s^{-1} and a temperature of 25 ± 1°C.

Ten randomly selected stomates were measured on each epidermal strip under bright field optics at a magnification of ×400 using an Olympus microscope fitted with a calibrated ocular micrometer. Only those stomates which appeared to have both guard cells in the same plane of view were selected. Sixty stomates were measured for each treatment except for the time course study (Fig. 2) when 45 stomates were measured for each point. All treatments lasted 3 h and experiments were repeated at least twice. On days when controls did not open normally in light or close in dark, data were discarded. Photomicrography was performed on a Zeiss microscope under bright field optics.

The Cyber Mainframe Computer at the University of Massachusetts was used in conjunction with Interactive Data Analysis Package to analyze the data. Where Anova indicated significant differences between means, the Student Newman-Keals Multiple

Range Test was performed.

All chemicals were supplied by the Sigma Chemical Company with the exception of KCl which was from the Fisher Scientific Company and Ethrel which was a gift of Dr. D. Green, University of Massachusetts.

RESULTS

The effects of IAA on the opening of *V. faba* stomates after 3 h in the dark are shown in Figure 1. There is a linear increase in stomatal aperture as IAA concentration increases logarithmically. With 0.01 mM IAA, stomates opened over 50% more than the control, while 1.0 mM IAA results in a greater than 100% increase in opening. All differences are significant from the controls at $P < 0.001$.

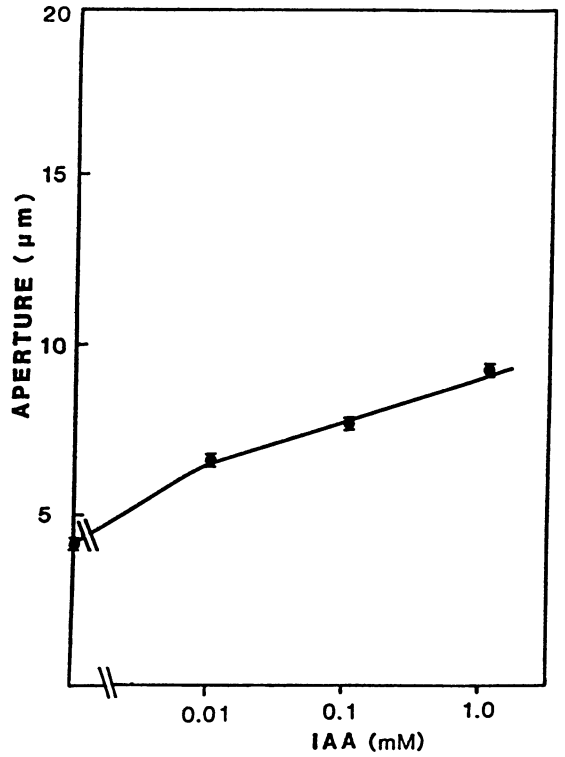

FIG. 1. Effect of IAA concentration on stomatal aperture. Epidermal peels were floated in the dark on solutions containing 10 mM Mes (pH 6.15) and 50 mM KCl for 1 h to close stomates. Peels were then transferred to test solutions containing 10 mM Mes, 50 mM KCl, and IAA for 3 h. Each point represents the mean of 60 measurements of individual stomata with SE.

Table I. *Effect of IAA and Ethrel in the Presence and Absence of AgCl on Stomatal Aperture under Light and Dark Conditions*

For light measurements, epidermal strips were peeled just prior to the start of the photoperiod and floated in the light on test solutions containing 10 mM Mes, (pH 6.15), 50 mM KCl, and either IAA (1.0 mM) or Ethrel (0.3% v/v) ± 0.14 mM AgCl. For dark measurements, the epidermis was peeled after the start of the photoperiod and floated in the dark on solutions containing 10 mM Mes and 50 mM KCl for 1 h to close stomates before transferring peels to the test solutions. Measurements represent the mean of 60 individual stomata ± SE.

	AgCl	Control	IAA	Ethrel
			μm	
Light	−	9.7 ± 0.2	11.1 ± 0.2	17.7 ± 0.4
	+	9.2 ± 0.2	9.2 ± 0.2	10.4 ± 0.3
Dark	−	3.9 ± 0.1	5.8 ± 0.2	10.0 ± 0.3
	+	4.0 ± 0.1	4.1 ± 0.2	4.4 ± 0.2

The stimulation of stomatal opening at high concentrations of IAA suggests a role for ethylene, since elevated levels of auxin promote ethylene synthesis (1, 12). To test this hypothesis, peels were incubated for 3 h in 0.1 mM IAA in the presence and absence of AgCl, an inhibitor of ethylene action (6). The effect of Ethrel (ethephon) was also tested on the peels with and without AgCl. Ethephon undergoes chemical decomposition at the higher pH of the cells, liberating ethylene as well as phosphonic acid and chloride ions. Experiments were performed under both light and dark conditions.

There is no effect of AgCl ($P < 0.001$) on stomatal apertures in the controls under both light and dark conditions (Table I, column 1). IAA (0.1 mM) stimulated opening in the dark by 2.0 μm over the control, while stomatal aperture in the light increases by 1.4 μm (Table I, column 2). However, this stimulation is prevented by AgCl. There is no significant difference ($P < 0.001$) between the control peels and those treated with both IAA and AgCl with or without illumination.

Peels incubated with Ethrel show a dramatic opening response in light (Fig. 2B) and dark (Fig. 2D) when compared with the controls (Figs. 2, A and C). Stomatal aperture is more than 2-fold larger than control peels in the light (an increase of 8 μm), while stomata in the dark open more than 6 μm over the controls (Table I, column 3). When AgCl is present, the opening response induced by Ethrel is almost eliminated; stomatal aperture increases only 0.7 μm in the light and 0.6 μm in the dark. This is not significant at the 0.05 level of probability for the former and at the 0.001 level for the latter.

The data in Figure 3 show that the difference between Ethrel-treated stomates and untreated controls is significant after only 30 min in either light or dark. Stomata in the light with Ethrel open maximally between 60 and 90 min and by 120 min without Ethrel. Stomata open maximally in the dark after 150 min in the presence of Ethrel. The small amount of opening observed in the dark control is probably due to the KCl (50 mM) in the incubation medium, but the size of the pore after 3 h is not significantly different from the 0-time size at the 0.05 level of probability.

DISCUSSION

Our results show that IAA is effective in opening stomates of *V. faba* in the light as well as in the dark. We suggest that this effect of IAA is due to ethylene for the following reasons: (a) the most effective concentrations of IAA for stomatal opening (Fig. 1) are those which are supraoptimal for growth, and high concentrations of IAA are known to stimulate ethylene evolution (1). (b) Figure 1 indicates a log-linear opening response to IAA, and ethylene production occurs linearly with the log of the IAA concentration (3). (c) Under our conditions, IAA increases stomatal aperture over controls within 30 min in the light (data not shown); similar kinetics are presented for Ethrel (Fig. 3). Ethylene production induced by auxin can be detected within 15 to 20 min after the addition of 0.25 mM IAA (9). (d) Ethylene derived from Ethrel can mimic the effects of auxin (Table I). (e) The effects of auxin are eliminated by AgCl, which is known to inhibit ethylene action (6). AgCl also eliminates the Ethrel-induced opening of *Vicia* stomates almost to control levels (Table I). Alternative explanations would implicate pH changes (10, 11) and/or the phosphonic acid and chloride liberated from Ethrel as promoting stomatal opening. It seems unlikely that AgCl could reverse opening caused by these agents; however, experiments with ethylene gas are needed to confirm these conclusions.

The extent of the ethylene effect is striking. Ethrel significantly stimulates opening by only 30 min, with maximal opening by 2 h to sizes twice that of the corresponding controls (Fig. 3). Such a rapid and pronounced effect of ethylene on stomates of *V. faba*

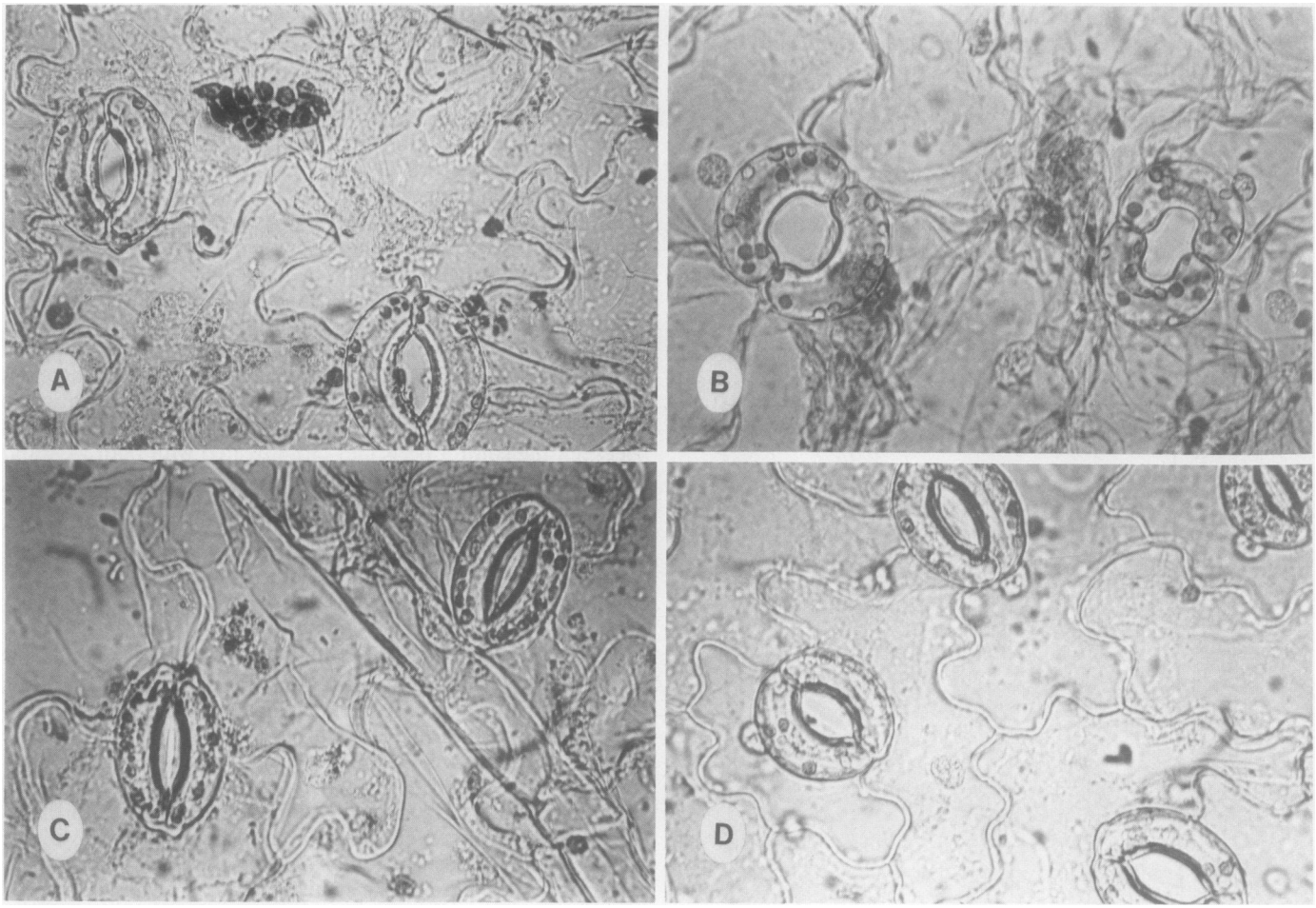

FIG. 2. Stomatal opening with and without Ethrel under light and dark conditions. A, light control; B, Ethrel-treated (0.3% v/v) in light; C, dark control; D, Ethrel-treated (0.3% v/v) in dark. Magnification, ×1375.

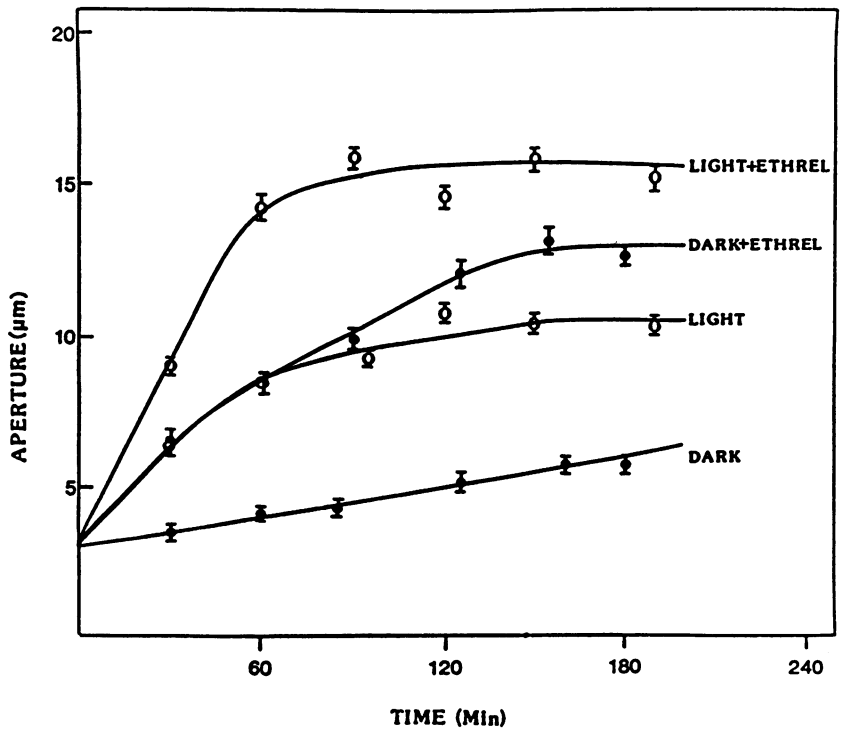

FIG. 3. Rate of stomatal opening with and without Ethrel under light (○) and dark conditions (●). Preparation of the peels is described in Table I. Means of 60 measurements of individual stomata are presented with SE.

makes this an ideal plant to study the mechanism of the gas on guard cell function and could also provide a means of studying the action of ethylene on membrane-localized events. The wide range of stomatal responses to auxin (14, 15, 19, 20, 26) and ethylene (16–18, 22, 23) may be characteristic of the species used by these workers. In this regard *V. faba* is unusual in having a highly active alkene oxygenase (2, 4) which converts ethylene to ethylene oxide. Thus, effective concentrations of ethylene could be rapidly reduced. This, plus many differences in methodology between the work of Tissera and Ayres (21) and this report may account for the paradox that in their hands ethylene appeared to close the stomates of *V. faba*.

Since added ethylene stimulates stomatal opening in *V. faba*, it is possible that, under certain conditions, ethylene evolved by the plant is responsible for changes in stomatal size. The opening by light probably does not involve ethylene, since the light response is unaffected by AgCl, IAA, or Ethrel (Table I). In fact, the data in Table I and Figure 3 show that the effect of Ethrel is close to additive with that of light.

However, 'stress' ethylene has been detected as a result of wounding or pathogen infection (1), and stomates of *V. faba* have been shown to open widely after infection by the pathogen, *Botrytis fabae*, even in the absence of illumination (24). Willmer (24) offers the possibility that enzymes, secreted by the pathogen, may be responsible for breaking down the cell walls of the guard cells, allowing their extension. It is also possible that a toxin secreted by the fungus affects the stomates similarly to fusicoccin (5, 21). However, the effect of fungal infection on the stomates in *Vicia faba* (Fig. 5.10 in Ref. 24) is strikingly similar to the effect of ethylene presented here (Fig. 2B). In both cases, stomates are opened abnormally wide. The possibility exists, therefore, that the effect of *B. fabae* on *Vicia faba* is mediated by stress ethylene.

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