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The Biochemical Response of Electrical Signaling in the Reproductive System of *Hibiscus* Plants[1]

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Stimulation of the stigma of *Hibiscus* flowers by pollen, wounding (heat), or cold shock (4°C) evokes electrical potential changes in the style, which propagate toward the ovary with a speed of 1.3 to 3.5 cm s^{-1}. Potential changes were measured intracellularly by microelectrodes inserted in the style. The resting potential ranged from −90 to −112 mV (n = 20) in cells of the vascular tissue and from −184 to −220 mV (n = 22) in cells of the pollen-transmitting tissue. The amplitude of the potential changes was between 40 and 150 mV, depending on the kind of stimulus. Self- as well as cross-pollination hyperpolarized the resting potential after 50 to 100 s, followed by a series of 10 to 15 action potentials. In contrast, cooling of the stigma caused a single action potential with a different shape and duration, whereas wounding generated a strong depolarization of the membrane potential with an irregular form and a lower transmission rate. To determine the physiological function of the different signals measured in the style, the gas exchange and metabolite concentrations were measured in the ovary before and 10 min after stimulation of the stigma. Self- and cross-pollination caused a transient increase of the ovarian respiration rate by 12%, which was measured 3 to 5 min after the stigma was stimulated. Simultaneously, the levels of ATP, ADP, and starch increased significantly. In contrast, both cold shock and wounding of the stigma caused a spontaneous decrease of the CO_2 content in the measuring chamber, as well as reduced metabolite concentrations in the ovary. Since the transport of labeled auxin from the top to the base of the style lasts at least 45 min, the influence of a chemical substance transmitted within 10 min is unlikely. Thus, our results strongly support the view that different, stimulus-dependent electrical signals cause specific responses of the ovarian metabolism.

Since the last century, electrical signaling in plants has been examined, and comprehensive reviews have been published by Pickard (1973) and Sibaoka (1966). An integral part of the generation of action potentials is based on changes in ion flux, including the efflux of chloride and potassium (Oda, 1976; Fromm and Spanswick, 1993) and the influx of calcium (Williamson and Ashley, 1982; Kikuyama and Tazawa, 1983; Beilby, 1984; Beilby and MacRobbie, 1984). In recent years, evidence was given that action potentials may regulate a wide variety of physiological responses in plants, including elongation growth (Shiina and Tazawa, 1986), respiration (Dziubinska et al., 1989), water uptake (Davies et al., 1991), phloem unloading (Fromm, 1991), activation of proteinase inhibitor genes (Wildon et al., 1992), and gas exchange (Fromm and Eschrich, 1993).

With regard to pollination, two different kinds of bioelectric potential changes have been observed in the style of flowers. First, Sinyukhin and Britikov (1967) measured an action potential in the style of *Lilium martagon* and *Incarvillea grandiflora* a few minutes after placing pollen on the stigma lobes. In addition, they measured an action potential only after mechanical irritation of the *Incarvillea* lobe that caused closure of the stigma lobes but was not propagated toward the ovary. However, in both species the pollen-induced action potentials propagated toward the ovary and stimulated the oxygen consumption by 5 to 11%, 60 to 90 s after the action potential arrived. This is probably the beginning of postpollination effects, such as the induction of enlargement of the ovary and wilting of the corolla, which occur long before fertilization. Second, Spanjers (1981) registered bioelectric potential changes that occur 5 to 6 h after pollination in the style of *Lilium longiflorum* flowers. The signals did not occur after application of killed pollen, which was heated at 150°C, or pollen of other species. To investigate the first reactions of the ovarian metabolism, we measured in this study various metabolites 10 min after stimulating the stigma by pollen, wounding, or cold shock.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

A yellow- and a red-flowering cultivar of *Hibiscus rosa-sinensis* L. (Malvaceae) were propagated by cuttings. The plants were grown in climate rooms under the following conditions: 28°C day/25°C night, 80% RH, and 250 μmol m^{-2} s^{-1} light intensity from 400-W mercury halide lamps for a 12-h photoperiod. Plants were irrigated with water at 14-h intervals and with a complete nutrient solution once a week. These growth conditions guaranteed flowering throughout the year.

For the experiments, the red-flowering cultivar was either pollinated with pollen of the yellow flowers (cross-pollinated) or with its own pollen (self-pollinated). Control experiments were made with dead pollen that was killed by heating in a Petri dish for 1 h at 150°C. The stigma lobes

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Abbreviations: 3PGA, glycerate-3-phosphate; SEM, scanning EM.

were always hand-pollinated with caution to prevent mechanical irritation during the measurements.

Measurement of Electric Potential Differences in the Style

Before the experiments started, the plants were placed in the Faraday cage (Science Products, Hofheim, Germany) for several hours at the same temperature, light intensity, and humidity as in the climate chamber. Electric potential changes were measured with glass microelectrodes with tip diameters of less than 1 μm, fabricated on a vertical electrode puller (model 700 C; David Kopf, Tujunga, CA) from 1.5-mm-wide borosilicate microcapillaries (Hilgenberg, Malsfeld, Germany), and back-filled with 100 mM KCl. The microelectrodes were clamped in Ag-AgCl pellet holders (World Precision Instruments, Sarasota, FL) and connected to microelectrode preamplifiers (input impedance 10^{12} Ω), to which a World Precision Instruments amplifier (model 750) was attached. The microelectrodes were inserted into the style using micromanipulators: electrode 1 at a mean distance of 5 mm below the stigma and electrode 2 at a mean distance of 10 mm above the ovary (Fig. 1, left). Both tips of the electrodes were either in one of the vascular bundles or in the transmitting tissue (Fig. 1, right). The arrival of the microelectrode tip in the measuring tissue was calculated from the potential and from microscopic examination. Since the diameter of the electrodes was less than 1 μm, we did not expect strong wound reactions that could cause ethylene production.

In contrast to the glass microelectrodes, the grounded reference electrode consisted of Ag-AgCl wire wrapped in moistened cotton (100 mM KCl) to provide appropriate contact with the plant surface just below the ovary. We zeroed the microelectrodes together with the reference electrode in 100 mM KCl solution before and after the measurements. This sort of measurement entails recording

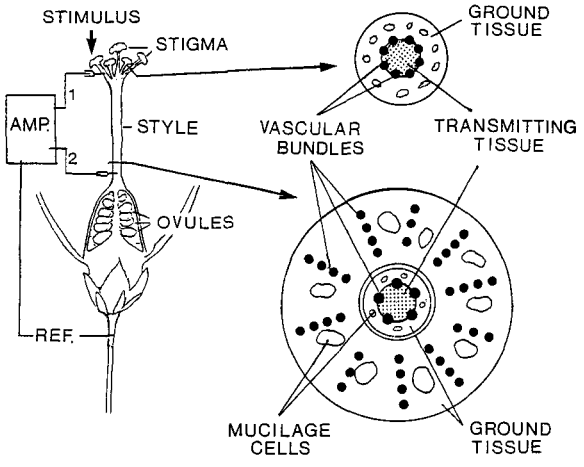

Figure 1. Left, Scheme of a *Hibiscus* flower for the measurement of electrical potentials in the style. AMP., Differential amplifier with channels 1 and 2; REF., reference electrode. The stigma was stimulated by pollen application, wounding, or cold shock (4°C). The style of the florescence covers the five-chambered ovary, and each chamber contains about 10 to 12 ovules. Right, Line drawing of cross-sections from the style at both electrode positions.

across a number of resistances and does not reflect the membrane electrical properties usually measured in isolated cells. Electric potential changes were generated by stimulating the stigma either with pollen or by wounding or cold shock.

Measurement of the Ovarian Respiration Rate

After the sepals and petals were removed, one ovary was enclosed by the measuring chamber (Fig. 2) to detect the CO_2 concentration with a porometer (Walz CQP-130, Effeltrich, Germany). Measuring conditions were 250 μmol m^{-2} s^{-1} light intensity, 25°C, and 60% RH. Then, the CO_2 amount (parts per million) was measured for approximately 1 h until it no longer showed fluctuations produced by cutting the floral leaves. After stabilizing, the stigma was stimulated with pollen, cold shock, or wounding, and the change of the ovarian respiration rate was detected during the whole experiment.

Metabolite Analysis in the Ovary

Flowers were quickly frozen in liquid nitrogen 10 min after the stigma was stimulated with pollen, cold shock, or wounding, taking care that they fell into liquid nitrogen immediately after being cut. Ovaries were isolated on dry ice to make extracts as follows. Phosphorylated intermediates, ATP, ADP, and AMP, were measured in TCA extracts as described by Hatzfeld et al. (1990). ATP was assayed in 100 mM Tris-HCl (pH 8.1), 0.25 mM $NADP^+$, 0.85 mM Glc, 0.56 unit of Glc-6-P dehydrogenase, and 0.7 unit of phosphoglucose isomerase, and 0.6 unit of hexokinase was added to start the reaction. The assay of ADP and AMP was in 50 mM Hepes (pH 7.0), 0.075 mM NADH, 1.6 mM PEP, and 4.4 units of lactate dehydrogenase, and 4 units of pyruvate kinase and 3.6 units of myokinase were added sequentially to start the reactions. A blank measurement was always carried out using 12 μM ATP in place of the extract to correct for small amounts of AMP, which can be present in commercial preparations of NADH.

Other glycolytic intermediates and soluble carbohydrates were analyzed as described by Stitt et al. (1989). For starch measurements, the sediment from the extraction was washed two times in 0.5 mL of ethanol and suspended in 0.8 mL of 0.2 M KOH. After incubation for 1 h at 95°C, the suspension was neutralized by 0.14 mL of 1 N acetic acid and centrifuged. Then, 45 μL of amyloglucosidase buffer was added to 5 μL of the suspension, which was incubated overnight at 37°C. The produced Glc was assayed as described by Stitt et al. (1989). All metabolite measurements were carried out using a Sigma ZFP 22 dual-wavelength photometer.

Histochemical Starch Determination

Longitudinal sections (15–20 μm thick and 0.3–0.7 cm long) of the ovaries were obtained by cutting with a cryostat (Jung, Heidelberg, Germany) at −10°C, according to the method of Krabel (1992). The sections were fixed and stored in a 1:3 solution of acetic acid and ethanol (96%). After the sections were washed in I_2KI for 30 s (Eschrich,

Figure 2. Arrangement for measuring ovarian gas exchange during stimulation of the stigma with pollen, cold shock, and wounding. CO_2 concentration was measured in parts per million with a porometer (Walz CQP-130) at 25°C and a RH of 60%. After the flower leaves were cut off, only the ovary was enclosed by the measuring chamber.

1976), they were enclosed in glycerin and photographed on a standard 18 photomicroscope (Zeiss).

Macroautoradiography

[^{14}C]IAA (specific activity = 47.2 mCi mmol^{-1}) was applied to the stigma at an activity of 10 μCi/flower, and after 10 and 30 min as well as 10 h the flowers were frozen with dry ice, freeze dried, and exposed on x-ray film as described by Eschrich (1966). In another series of experiments the stigma was removed from the style, and [^{14}C]IAA was applied at 10 μCi to the cut surface at the top of the style. The flowers were harvested after 15 and 45 min and prepared for macroautoradiography like the unwounded ones.

SEM Observations

For SEM observations, small pieces of pollinated stigma were freeze dried and coated by carbon gold. An electron microscope (Philips 515) was utilized at 15 kiloelectron volts.

RESULTS

Anatomy and Resting Potentials of the Style

In introductory experiments, the anatomical structure of the style was examined microscopically and resting potentials of cells from different tissues were measured. First, the diameter of the style increases in a basipetal direction (Fig. 1, right). The pollen-transmitting tissue in the center of the style connects the stigmatic tissue with the ovules and serves as a path for the growing pollen tube and as a source of nutrients (Kroh et al., 1970). It is embedded in the ground tissue and associated with the vascular bundles. At the top of the style the cross-section through a branch 5 mm below the stigma shows that the transmitting tissue is enclosed by one core of ground tissue (Fig. 1, top right). In contrast, two cores of ground tissue surround it at the base of the style (Fig. 1, bottom right). In this area the transmitting tissue is always associated with five vascular bundles, whereas the thick outer core of ground tissue contains 10 rows of three to four vascular bundles. Mucilage cells, which are typical for *Hibiscus* plants, occur in both inner and outer ground tissue.

Second, resting potentials were measured in the ground tissue and the vascular as well as transmitting tissue after the reference electrode was attached to the surface below the ovary. The potential values consistently occurred in two distinct categories: between −90 and −112 mV (n = 20) in the vascular tissue and between −184 and −220 mV (n = 22) in the pollen-transmitting tissue. In contrast, the values of the ground tissue showed higher deviations and did not fall into a category, probably because of the frequent distribution of mucilage cells in this tissue. The position of the microelectrode tip could be calculated from the value of the potential that correlated with the penetration distance between the site of electrode entry and the measuring tip. Measurements were first made 5 mm below the stigma, because of the uniform anatomical structure of this area. The penetration depth of the electrode was visible on the micromanipulator scale and was checked microscopically after the measurements.

Bioelectric Potential Changes in the Style

Figure 3 illustrates electric potential changes in the style in response to pollen application, wounding, and cold shock. Both electrodes inserted in the vascular tissue of the style yielded the same signals after stimulating the stigma and were used to calculate the transmission velocity. For simplicity, only the recordings of one electrode are shown. The resting potential of the measured vascular cells was between −90 and −112 mV, and the amplitude of the potential changes was between 40 and 150 mV, depending on the kind of stimulus. Most of the style cells are electrically coupled by plasmodesmata to provide signal conduc-

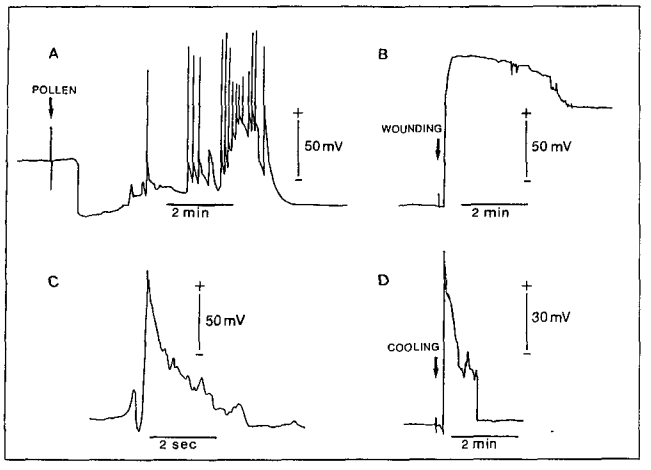

Figure 3. A, Electrical recording in the vascular tissue of the style in response to live pollen application. B, Electrical potential change in response to wounding the stigma with a burning joss stick. C, Typical action potential caused by pollination (taken from A with enlarged time scale). D, Action potential in response to cooling (4°C) the stigma. Traces are from electrode 2 at the position 10 mm above the ovary.

tion. Self- and cross-pollination hyperpolarized the resting potential after 50 to 100 s, followed by a series of 10 to 15 action potentials (Fig. 3A), which propagated with a velocity of 3.5 cm s^{-1} toward the ovary (data not shown). In Figure 3C it is shown that one of these action potentials has a duration of approximately 2 s. After signal transmission the resting potential remained stable at values greater than −140 mV. No electric potential change was measured after application of killed pollen.

In contrast to pollination, wounding the stigma with a burning joss stick caused a strong depolarization of the membrane potential, which had an irregular form (Fig. 3B) and a transmission velocity of 1.3 cm s^{-1}. The original resting potential of −90 to −112 mV was not reestablished, indicating that the voltage change is not an action potential. As in animal cells, an action potential is defined as a propagating, transient change in voltage with an all or none response, which does not usually depend on the strength of the stimulus. With regard to pollination, a series of 10 to 15 action potentials can be evoked by one pollen grain and is independent of the amount of pollen.

Another type of signal was generated when the stigma was stimulated by cold shock with a decrease of water temperature (4°C), which is also known to evoke the generation of action potentials (Pickard, 1973; Jones and Wilson, 1982). This treatment generated a single action potential with a duration of approximately 1 min (Fig. 3D), which was transmitted with a speed of 2 cm s^{-1} toward the ovary. Thus, the transmission velocity of the different signals was similar (1.3–3.5 cm s^{-1}), whereas the number, duration, and shape changed according to the kind of stimulus. It should be mentioned that propagating electric signals could also be measured in cells of the pollen-transmitting tissue with similar properties as in vascular cells. In contrast, cells of the ground tissue were not excitable.

Transmission of Chemical Messengers

To determine the transmission rate of a chemical substance from the stigma to the ovary, ^{14}C-labeled IAA was applied to the stigma and translocation was recorded by macroautoradiography. Figure 4 (top) shows three unwounded flowers on which [^{14}C]IAA was applied to the stigma for 10 min, 30 min, and 10 h before the flowers were freeze dried. The experiments were prolonged up to 10 h because radioactivity needed that long to be transported to the base of the style (arrow). It is interesting that only the inner part of the style appeared labeled. To test whether the slow transport is caused by a delayed uptake of auxin at the stigma, the latter was removed and labeled IAA was applied to the cut surface at the top of the style. The macroautoradiographs indicated that label spreads with a velocity of 1 to 2 mm min^{-1} toward the ovary (Fig. 4, bottom), which is faster than the transport in intact styles but slower than electrical signaling. Since the outer part of the style appeared labeled, auxin also was transported in this part. So far we have not tested whether the phloem is responsible for the transport, but microautoradiographic studies are planned to investigate this. In conclusion, the results provide evidence that a chemical messenger cannot

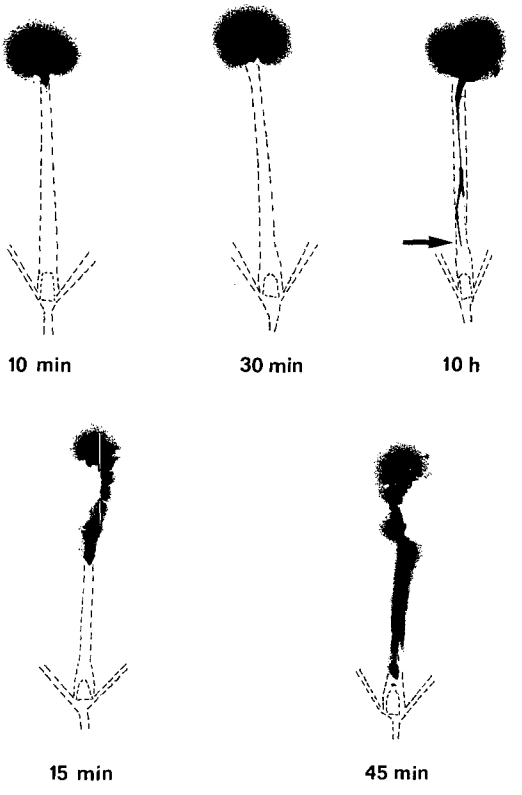

Figure 4. Top, Macroautoradiographs of three styles that had been treated with [^{14}C]IAA at the stigma for 10 and 30 min as well as 10 h. Transport of label occurs only in the inner part of the style and needs 10 h to reach its base (arrow). Below, Macroautoradiographs of styles with removed stigma. Labeled IAA was applied on the cut surface at the top of the style and arrived at the base after 45 min (right style), showing that the outer style part is also involved in transport.

be transported from the top to the base of the style within 10 min.

The Effect of Stimulation on the Ovarian Rate of Respiration

To investigate a possible physiological function of the electrical signals in the reproductive system, the ovary was placed in a chamber for gas-exchange measurements (Fig. 2). As shown in Figure 5, the CO_2 concentration in the measuring chamber changed soon after stimulation of the stigma. Pollination caused a transient increase of the ovarian respiration rate by 12%, which was measured 3 to 5 min after stimulation. In contrast both cold shock (cooling the stigma with ice water) and wounding by heat caused a spontaneous decrease of the CO_2 content in the chamber (Fig. 5). In the case of cooling, the respiration rate was reestablished after 30 min (data not shown), which did not occur after wounding. Thus, the course of changes in the rate of respiration depends on the character of the stimulation. If stimulation of the stigma does not produce an electrical signal, e.g. during touching the stigma, no respiration change occurs in the ovary.

The pollination-induced increase of respiration corresponds to previous studies in orchid flowers (Hsiang, 1951). Goh et al. (1982) reported that much of the increase in *Vanda* flowers is centered in the placenta and exhibits three peaks, one of which occurs immediately after pollination.

Metabolites and Carbohydrates in Ovaries of Unstimulated and Stimulated Flowers

To determine whether the changes of the respiration rate are connected to changes of ovarian metabolism, the concentrations of various metabolites were measured in ovaries 10 min after stimulation. In Table I it is shown that ovaries of pollinated flowers have slightly higher ATP levels (+12%) than those of untreated flowers, whereas cold-shocked and wounded flowers have lower concentrations (−19 and −22%, respectively). These differences confirm that the respiration rate increases after pollination, whereas it decreases after cold shock and wounding. The ADP concentration, which is about 3-fold less than ATP in ovaries of untreated flowers, correlates with the changes of the ATP level. After pollination it increased significantly (+37%) when compared with untreated flowers. However, after stimulation by cold shock and wounding, ADP decreased in the range of 20 and 9%, respectively. In contrast, the amount of ovarian AMP appeared to be much less than ATP and ADP and changed only significantly after pollination (−43%).

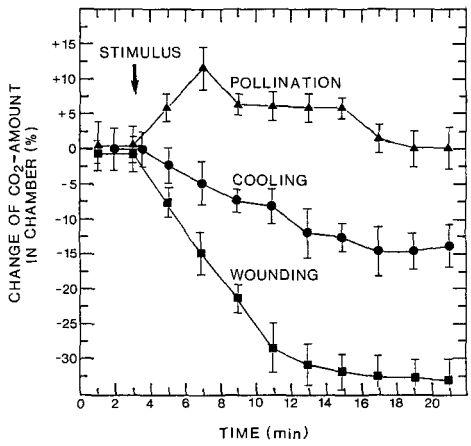

Figure 5. Changes of the ovarian respiration rate after stimulation of the stigma with pollen, cold shock, and wounding. CO_2 concentration changes were transformed into percentages. Data are the mean values ± SE from 10 ovaries. Initial [CO_2] was the mean ± SE.

Table I. *Adenine nucleotide levels of ovaries*

Flowers were frozen in liquid nitrogen 10 min after the stigma was stimulated with pollen, cold shock, or wounding. Ovaries were isolated to make extracts for the measurements. The results are the means ± SE from 10 ovaries.

Flower Treatment	ATP	ADP	AMP
	nmol g^{-1} fresh wt		
Untreated	292 ± 24	90 ± 11	16 ± 3.0
Pollinated	328 ± 28	124 ± 14	9.0 ± 2.0
Cold shocked	235 ± 21	72 ± 8.0	12 ± 4.0
Wounded	228 ± 23	82 ± 9.0	18 ± 4.0

Second, in Table II it is shown that the levels of Glc, Fru, and Suc are higher in pollinated flowers than in untreated ones (Glc, +10%; Fru, +21%; Suc, +8%). Most striking is a 15-fold increase of the starch concentration, which does not change significantly after cold shock but increased 4-fold after wounding.

However, the latter treatment reduces the Suc level drastically (−51%). Since Suc is the main sugar transported via the phloem into growing flowers, the differences imply that Suc import might be blocked after wounding. This is also valid for cold-shocked flowers, in which Suc decreased in the range of 49%. In contrast, the Glc and the Fru levels showed only slight differences after cold shock and wounding.

Since the concentrations of starch, ATP, and hexoses clearly increased after pollination, the concentrations of Glc-1-P, which is a necessary substrate for starch synthesis, and other glycolytic metabolites also were measured (Table III). In contrast to Glc-1-P, which increases after pollination (+41%), other hexose phosphates (Glc-6-P, Fru-6-P) do not change much. A little decrease of UDP-Glc (−16%) and

Table II. *Carbohydrate levels of ovaries 10 min after different treatments of the stigma*

The results are the means ± SE from 10 ovaries.

Flower Treatment	Glc	Fru	Suc	Starch
	nmol g^{-1} fresh wt			*mmol Glc g^{-1} fresh wt*
Untreated	643 ± 56	573 ± 43	368 ± 27	0.37 ± 0.05
Pollinated	709 ± 61	698 ± 65	397 ± 37	5.80 ± 0.7
Cold shocked	590 ± 57	506 ± 49	186 ± 20	0.32 ± 0.04
Wounded	646 ± 62	492 ± 48	180 ± 19	1.69 ± 0.2

Fru-6-P (−5%) might be correlated with the slight increase of Suc (+8%) and indicates an activation of Suc synthesis.

Most of the measured metabolites clearly decrease after cold shock and wounding: UDP-Glc (−29%), Glc-6-P (−34%), and Fru-6-P (−24%) decreased after cold shock and showed changes of −41, −43, and −42%, respectively, after wounding the stigma. Other glycolytic metabolites show similar characteristics; they are drastically diminished after cold shock (3PGA, −88%; PEP, −86%; pyruvate, −71%) and also significantly after wounding and pollination of the stigma.

Distribution of Starch in Unstimulated and Stimulated Ovaries

Ovaries from unpollinated and 10-min-pollinated flowers were quickly frozen in liquid nitrogen, and longitudinal sections were stained with I_2KI for histochemical starch determinations. Detailed microscopic observations showed that in unpollinated flowers only the outer wall of the ovary and some of the embryo sacs (Fig. 6, A and B) of the ovules accumulated starch-filled amyloplasts. In contrast, most of the embryo sacs and the upper central placenta of ovaries from pollinated flowers contained strong starch pools, which stained black (Fig. 6, C and D). According to the quantitative starch determinations, ovaries from cold-shocked flowers showed the same distribution as those from unpollinated ones, whereas ovaries from wounded flowers appeared similar to pollinated ones. After wounding they also accumulated starch in the placental tissue but not in the embryo sacs of the ovules.

SEM Observation of the Pollinated Stigma

To investigate the mechanism of stimulus reception as a first approach, we observed the surface of the pollen and the stigma by using SEM. Figure 7A shows several pollen grains on the papillae of the stigma, which are connected to each other by pollen kit. The question of how a pollen grain stimulates the papillae cells of the stigma to produce an electric potential change cannot be answered. A possible mechanism might be the opening of ion channels in the plasmalemma of the stigma induced by pollen. Based on patch-clamp investigations, Edwards and Pickard (1987) observed mechanotransductive ion channel activity in the plasmalemma of tobacco protoplasts. However, in *Hibiscus* the train of 10 to 15 action potentials after pollination is difficult to explain by mechanically stimulated currents across the plasmalemma, since dead pollen does not evoke potential changes but causes the same kind of pressure. Also, touching the stigma does not produce an electrical signal. On the contrary, excitation might be evoked in the plasmalemma of the papillae cells by a chemical substance that is secreted by the pollen, which shows many apertures on the surface (Fig. 7B). This hypothesis also explains why killed pollen, which also generates pressure, does not induce an electrical response. Thus, the analysis of substances secreted by pollen grains and their effects on membrane excitability might help to explain the phenomenon of signal generation at the stigma. With regard to pollen of orchids, auxin was found to be present (Arditti, 1971a, 1971b) and evidence supported the idea that auxin spreads slowly from the stigma to other parts of the flowers to stimulate the production of ethylene (Burg and Dijkman, 1967), which leads to floral fading. So far we have only investigated the transport of auxin in *Hibiscus* styles but have not measured auxin concentrations in pollen grains and their effects on electrical signaling.

DISCUSSION

Our observations on intact, plant-attached *Hibiscus* flowers support the hypothesis of a stimulus-dependent generation of different electrical signals in the stigma and style, with specific influences on the ovarian metabolism. The assumption that electrical signaling caused the biochemical response in the ovary was confirmed by autoradiographs showing that ^{14}C-labeled auxin applied to the stigma or the cut surface of the apical style needed at least 0.75–10 h to be transported to the ovary (Fig. 4).

Furthermore, there exists a "theoretical"" argument that chemical signals cannot travel at the observed rate. Given the transmission rate (10 cm/10 min = 0.017 cm s^{-1}), the diffusion coefficient (D) of a putative chemical signal would be (according to the Einstein random walk equation):

$$D = d^2/2t = (0.017)^2/2(1) = 1.4 \times 10^{-4}\ \text{cm}^2/\text{s}$$

$$= 1.4 \times 10^{-8}\ \text{m}^2/\text{s}$$

where t is time.

Using the Stokes-Einstein equation, we can predict the radius (r) of a spherical molecule that has such a diffusion coefficient:

$$D = kT/6\pi r\eta \text{ or } r = kT/D6\pi\eta$$

Table III. *Levels of UDP-Glc, Glc-6-P, Fru-6-P, Glc-1-P, 3PGA, PEP, and pyruvate in ovaries 10 min following stigma stimulation*

The results are the means ± SE from 10 ovaries.

Flower Treatment	UDP-Glc	Glc-6-P	Fru-6-P	Glc-1-P	3PGA	PEP	Pyruvate
				nmol g^{-1} fresh wt			
Untreated	63 ± 7.0	302 ± 28	83 ± 7.0	34 ± 4.0	50 ± 5.0	30 ± 2.0	14 ± 1.2
Pollinated	53 ± 6.0	310 ± 29	79 ± 6.0	48 ± 5.0	13 ± 2.0	15 ± 2.0	7.0 ± 0.8
Cold shocked	45 ± 5.0	197 ± 20	63 ± 6.0	38 ± 4.0	6.0 ± 1.0	4.0 ± 0.5	4.0 ± 0.3
Wounded	37 ± 4.0	170 ± 19	48 ± 5.0	30 ± 3.0	21 ± 2.0	12 ± 1.0	9.0 ± 1.1

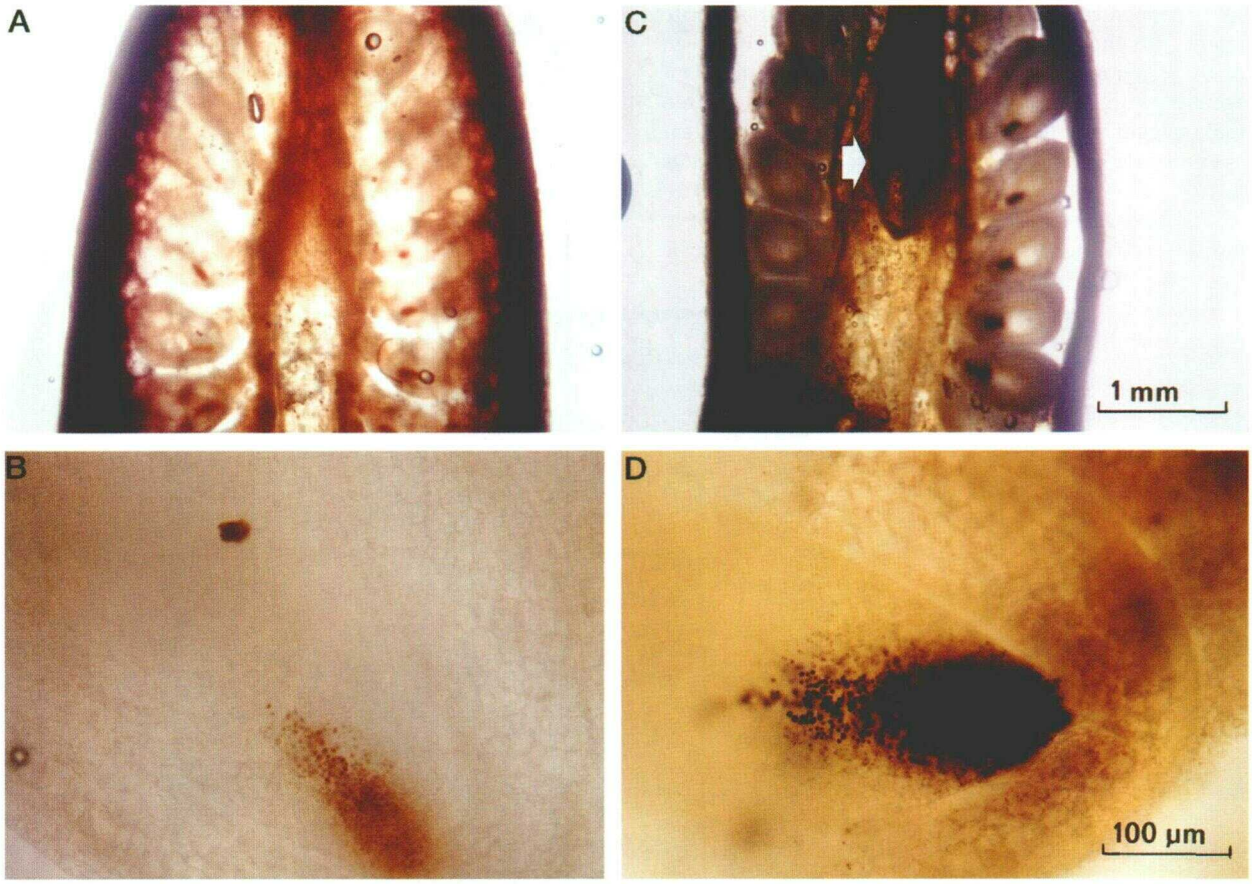

Figure 6. The qualitative starch reaction with I_2KI shows the distribution of starch in ovaries and ovules of unstimulated flowers (A and B) and pollinated ones (C and D). A, Longitudinal section of the unstimulated ovary that was quickly frozen in liquid nitrogen before sectioning. Starch accumulated in the outer ovary wall, which stained black. B, Some starch-filled plastids accumulated in the embryo sac. C, Longitudinal section of the ovary frozen 10 min after the stigma was pollinated. The upper region of the central placental tissue shows an area extremely saturated with starch (white arrow). D, The embryo sac of the ovules from pollinated flowers, which also accumulated a large amount of starch-filled amyloplasts.

where k is Boltzmann's constant, T is absolute temperature, and η is viscosity (Pa s). If $T = 298$ K, $\eta = 0.001$ Pa s, and $k = 1.38 \times 10^{-23}$ J/K, then $r = 16 \times 10^{-12}$ m.

Since this is about the radius of a single carbon ion, it is unlikely that any molecule can diffuse so quickly. Thus, the message must travel from the stigma to the ovary by electrical signals.

So far, our results confirm the measurements of Sinyukhin and Britikov (1967), who found an increase in the ovarian respiration rate minutes after pollination and electrical signaling in *Lilium* and *Incarvillea* flowers. Based on gas-exchange measurements, metabolite analysis, and histochemical investigations, now we can provide evidence that the ovarian metabolism responds quickly in contrary ways to different stimuli.

First, the pollination-induced increase of the respiration rate seems to be in good agreement with the increase of the ATP concentration. Clearly, this supports the view that the female reproductive system prepares for the following fertilization by increasing its metabolism. Most surprising is the 15-fold increase of the starch level 10 min after pollination. Furthermore, the histochemical investigation shows that starch is synthesized mainly in cells of the upper central placenta (Fig. 6C, white arrow) but also clearly in cells of the embryo sacs of the ovules. Obviously, the latter starch accumulation should provide carbohydrates for embryonic growth. This pattern is in agreement with the observation that ovules from cold-shocked and wounded flowers cannot be distinguished from ovules of unpollinated ones, because the egg cell will not be fertilized and the embryo will not develop. A slight increase of Glc-1-P and Glc, which can be used unsplit for starch synthesis in amyloplasts of storage tissues, indicates, too, that starch synthesis is dominant after pollination, whereas decreasing levels of 3PGA, PEP, and pyruvate reveal a decrease of glycolysis. However, since the starch level increases much more than the other carbohydrates, the hexoses will be needed to produce starch and much more Suc has to be imported via the phloem into the ovary.

Table II shows that the Suc concentration increased slightly after pollination to provide more storage material. In this context, Wang and Fisher (1994a, 1994b) investigated the phloem-unloading process in attached wheat grains and found a continued import of Suc into grains

against its concentration gradient. They suggest that solute movement into the grain might occur by pressure-driven flow or by active membrane transport. As shown in recent experiments with *Mimosa* pulvini (Fromm, 1991), action potentials trigger phloem unloading of Suc during the leaf movement cycle. Furthermore, from orchid flowers it is known that after pollination an increase in movement of minerals and carbon into the actively growing embryo region occurs (Arditti, 1979). Thus, the slight increase of the Suc level after pollination of *Hibiscus* flowers might also be caused by a Suc import into the ovary via the phloem, which is necessary to produce starch in the measured dimension.

Second, with regard to cold-shocked flowers, the levels of almost all metabolites decreased (with the exception of starch and Glc-1-P), indicating a transient metabolic reduction, which correlates with the reduction of the respiration rate (Fig. 5). In contrast, wounded flowers showed a stron-

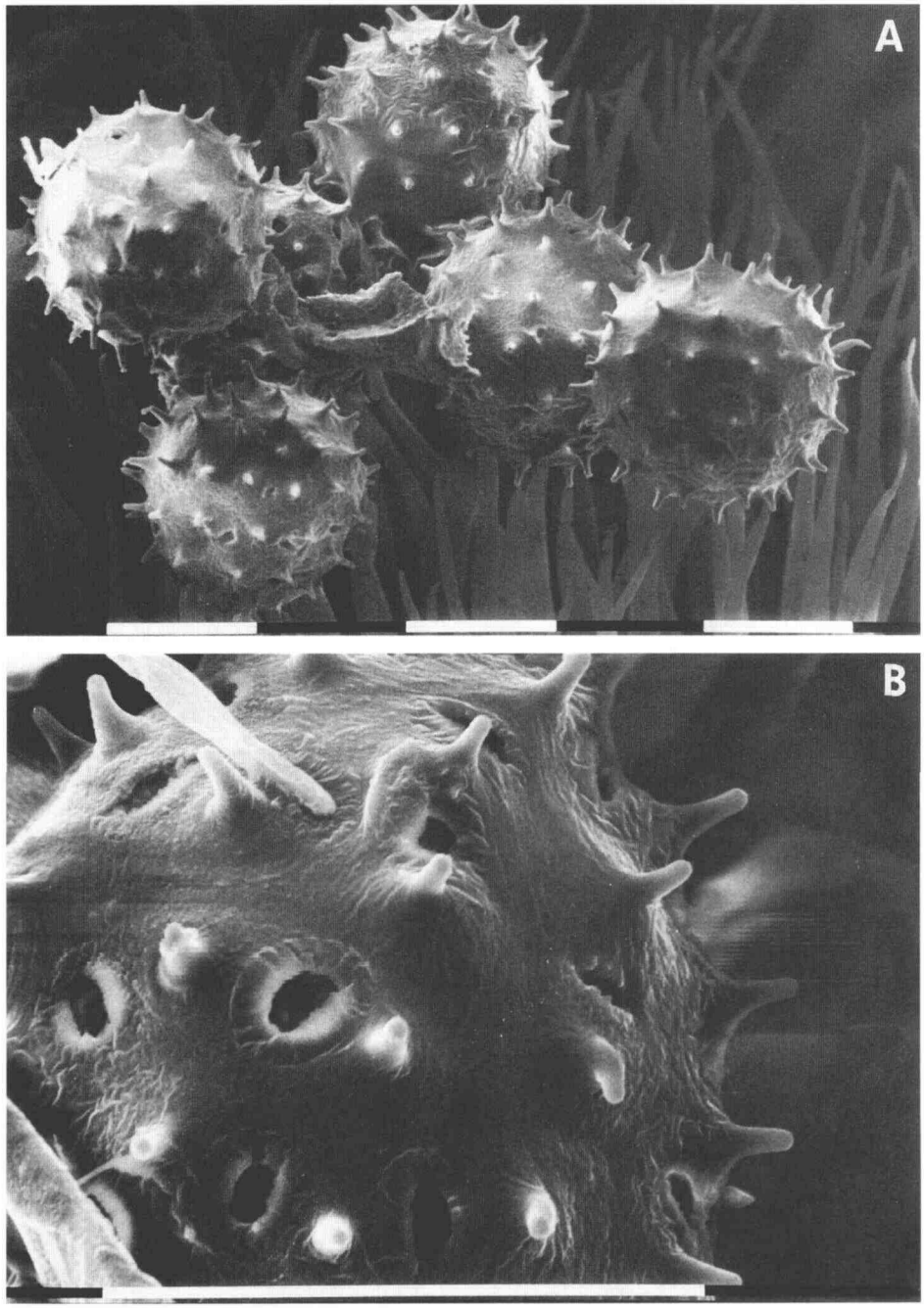

Figure 7. A, SEM photograph of pollen grains that adhered to the papillae of the stigma. The papillae facilitate pollen reception and respond to pollen with excitation. B, Detailed view on the surface of a pollen grain showing appendages that facilitate adherence to the stigmatic papillae (above on the left). The photograph also shows a pattern of apertures, which are able to secrete substances. White bars = 0.1 mm.

ger reduction of the respiration rate, which remained low for hours. In good agreement are the reduced levels of almost all metabolites with the exception of AMP, Glc, and starch. Since the latter increased 4-fold after wounding, we suggest that starch is probably needed for wound-repair reactions in the ovary. However, only the stigma was wounded 8 to 10 cm from the ovary. With regard to wound reactions, it also has been shown that membrane properties do change in leaves that are distant from those wounded, resulting in the formation of more fragile protoplasts (Walker-Simmons et al., 1984). Other authors (Gaspar et al., 1985) suggested that stress-induced membrane depolarization leads to generation of free radicals and peroxides, which cause breakdown of membrane lipids. Recent work on maize plants has also shown that membrane properties do change in leaf parts that are distant from those stimulated, expressed in a reduced phloem transport (Fromm and Bauer, 1994).

To conclude, our work has shown that different stimuli applied to the stigma of flowers evoke specific electrical signals that propagate toward the ovary. Since the transmission of a chemical substance within 10 min over a distance of 8 to 10 cm from the stigma to the ovary could not be proven, the metabolism responded to the electrical signals. In light of these results the question arises how an electrical signal is able to cause the biochemical response. Since communication between the stigma and the ovary is achieved through the propagated changes in membrane potential, the biochemical response might be achieved through the subcellular changes of K^+, Cl^-, and Ca^{2+} ions. Davies (1987) reported that local changes in ion concentration can lead to modified activities of enzymes in the cell wall (e.g. pectinase), the plasmalemma (e.g. cellulose synthetase), and the cytoplasm (e.g. protein kinase). A similar mechanism might also be responsible for the fluctuation of the starch level in ovaries. Extensive investigations have shown that the biochemical regulation of starch synthesis is centered almost exclusively on ADP-Glc-pyrophosphorylase (Preiss et al., 1985). Thus, further analysis of starch synthesis in ovaries will take the characteristics of this enzyme into consideration to provide insight into the biochemical role of electrical signaling.

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