Short Communication

On the Involvement of Acetylcholine in Phytochrome Action

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In a recent publication Jaffe (1) presented data which implicated acetylcholine as the causative agent in the phytochrome-mediated attachment of mung bean root tips on a glass surface. Since ACh (1) is responsible for the bioelectric potential changes in certain nerve cells of animals, proof of its participation in any phytochrome-mediated process is of great significance in elucidating the mechanism of phytochrome action. In this study, attempts have been made to confirm Jaffe’s finding that ACh mimics the effect of red light in causing root tips to adhere to glass after far red irradiation.

MATERIALS AND METHODS

The materials and methods used have been reported (2). In brief, mung bean seeds (Phaseolus aureus L., var. Oklahoma 612) were soaked for 8 hr in darkness. They were then exposed to 8 min of far red light (190 μW cm⁻² from 700–750 nm) and allowed to germinate in darkness over deionized water. All operations were done at 24 °C. Three days later, 10 root tips of 2 mm length were excised in dim light and placed in a beaker with 10 ml of solution of the following composition: KCl, CaCl₂, and MgCl₂ of 0.1 mM; MnCl₂, 2 μM; L-ascorbic acid, 1 μM, and 3-IAA, 0.2 mM. To this solution ACh chloride was added to a concentration of 0.3 mM.

The beaker was immediately placed on a phonograph turntable in a beam of red light (4.0 μW cm⁻² from 650–680 nm) with the root tips arranged 2.5 cm from the center of the turntable. Once every minute during 5 min exposure to red light, attachment of root tips was assayed by gradually rotating the turntable to a speed of 33 rpm. This speed is adequate to dislodge any root tip that has a more negative surface bioelectric potential following far red irradiation. After red light exposure, the root tips were irradiated with far red light (8.9 μW cm⁻² from 710–750 nm) for 7 min during which the number of attached root tips was scored once every minute. The cycle was repeated at least once. Each experiment was repeated more than six times with different lots of seedlings.

RESULTS AND DISCUSSION

It has been reported earlier (2) that far red irradiation of hydrated mung bean seeds results in seedlings with root tips showing poor attachment to glass in red light. The root tips in this study reveal similar behavior. Some typical results from one experiment are presented in Figures 1 and 2. In the absence of ACh, root tips show slight attachment in red light and none in far red light (Fig. 1a). Addition of ACh to the solution to a concentration of 0.3 mM causes the root tips to adhere to the glass in both red and far red light (Fig. 1b). These results confirm Jaffe’s findings that ACh induces root tip attachment. However, since data not reported here indicate that ACh has little or no effect on attachment at concentrations below 0.1 mM, it appears unlikely that ACh is acting in this case as a hormone or transmitter. The fact that it is effective only at relatively high concentrations indicates that its mode of action could be by interfering with one of the actions essential for the process (Jaffe used a concentration of 5 mM). This possibility was explored by increasing the concentrations of the other cations in the assay solution. When

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2 Abbreviation: ACh: acetylcholine.
the K+ concentration is increased to 1 mM after the first cycle, the attachment induced by ACh is eliminated (Fig. 1c). The root tips now show little or no attachment in red or far red light. Increasing the concentrations of either Mg2+ or Ca2+ does not reverse the effect of ACh (Fig. 2a). Neither does the addition of Rb+ to 1 mM remove the ACh-induced attachment (Fig. 2b). These results demonstrate the absolute requirement for K+ in the detachment process. Attachment promoted by ACh is apparently not unique. For instance, when Na+ is added to the medium to a concentration of 0.3 mM, the root tips adhere in both red and far red light just as they do with ACh (Fig. 2c). In this case also, a higher concentration of K+ reverses the induced attachment.

The results presented in Figures 1 and 2 clearly illustrate that the attachment of root tips to glass brought about by ACh is probably through its behavior as a cation interfering with the function of K+ in the detachment process and not as a hormonal agent of phytochrome. It is possible that ACh competes for some essential K+ sites on the plasmalemma. By occupying these sites, it could prevent K+ from bringing about the necessary bioelectric potential changes which result in detachment of root tips.

Since no attempt has been made in this study to determine the endogenous level of ACh in root tip cells, these results do not rule out the other finding of Jaffe (1) that ACh is released during red light irradiation of mung bean root tips.

LITERATURE CITED
