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Short Communication

Promotion of Cell Elongation in *Avena* Coleoptiles by Acetylcholine[1]

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It is well established that acetylcholine acts as a regulator of cell membrane permeability at the synapse of nerve cells (2), as well as in other types of animal cells (12, 20). There is mounting evidence that ACh[2] also occurs naturally in a variety of plants (1, 8, 10, 11, 19). Recently, Jaffe (11) has shown that acetylcholine is capable of mimicking the effect of red light in inducing the adhesion of mung bean root tips to a negatively charged glass surface. He has presented evidence that this might result from an ACh-mediated increase in the efflux of H^+ or other cations from root tip cells and has suggested that ACh may act as a regulator of membrane permeability in plant cells as it does in animal cells.

We have previously suggested on the basis of indirect evidence that the very rapid promotion of cell elongation by auxin and by certain other agents may involve a sudden change in the properties of cell membranes involved in communication between the cytoplasm and the cell wall of growing plant cells (5–7, 17). We have therefore investigated the growth-regulating ability of ACh and the influence of ACh on the action of indole-3-acetic acid in the promotion of cell elongation.

MATERIALS AND METHODS

The experiments were done using 8-mm segments taken beginning 2 mm below the tip of 3-day-old etiolated oat coleoptiles (*Avena sativa* L., var. Victory). The oats were grown as described in ref. 6. Each experiment was done using a vertical column of 14 of these segments immersed in the appropriate growth medium. The device used for following the combined elongation of the tissue segments is a modified version of the shadowgraphic recording apparatus described in detail in ref. 6, and all the curves shown in this work are direct tracings of original continuous shadowgraphic records obtained as described in that reference. Since the magnification differs slightly for each record, a marker representing 1 mm increase in length of the entire column of segments is shown at the end of each curve.

The growth medium surrounding the coleoptile segments was continuously gassed with oxygen and was buffered at pH 6.8 with phosphate buffer. All experiments were done under dim red light at 26 C.

RESULTS AND DISCUSSION

Figure 1 compares the response of coleoptile segments to IAA (Baker Chemical Co.) and to ACh chloride (Sigma Chemical Company). The response to IAA shown in curve A exhibits a latent period of about 10 min which is typical for coleoptile tissue (5, 6). Curves B and C show that ACh is also capable of causing an acceleration of the rate of elongation in this tissue. However, the response to 10 μM ACh (curve C) is only about 15% as great as the response to the same concentration of IAA. A 10-fold higher concentration of ACh (curve B) supports a stronger growth response but still only about 25% as great as the response to 10 μM IAA. In both of these experiments the latent period in response to ACh was about 20 min, *i.e.*, about twice the length of the latent period in response to auxin. Curve B also shows that the rate of elongation in the presence of ACh can be further increased by adding auxin (second arrow).

When segments elongating in the presence of IAA were treated with a medium containing both IAA and ACh, no response to the added ACh was observed during the subsequent 3 hr (first 20 min only shown in Fig. 1, curve A).

Figure 2 shows that growth promotion by ACh can be completely prevented by brief pretreatment of the tissue with 1 mM KCN. This strong sensitivity to a metabolic inhibitor distinguishes growth promotion by ACh (and by IAA, see ref. 6) from growth promotion by certain other treatments that exhibit low sensitivity to metabolic inhibitors (7, 16). The inhibition by KCN appears to be completely reversible since withdrawal of KCN leads to an increase in the rate of elongation to a value typical of that obtained after treatment with ACh alone. However, this interpretation is complicated by the observation that application and withdrawal of cyanide in the absence of ACh also causes a substantial promotion of the rate of elongation (curve B) as reported earlier by Ray (15).

Since atropine is known to inhibit the neurohumor activity of ACh in animals, most likely interfering with ACh at its site of action (9), the effect of atropine sulfate (Merck) on ACh-promoted cell elongation was also tested. Figure 3 shows that 0.1 mM atropine sulfate very quickly inhibits growth in response to ACh. Curve A in Figure 3 shows that inhibition by atropine sulfate is somewhat specific since growth in response to IAA is not inhibited by this alkaloid. These results in conjunction with the observation that growth promotion by ACh is weaker and is slower to develop than growth promotion by IAA, make it unlikely that ACh plays a role as a mediator of auxin action in the promotion of cell elongation.

Figure 4 shows that ACh is capable of rapid but incomplete reversal of the inhibition of auxin-promoted cell elongation by 10 mM calcium chloride. Since displacement of Ca^{2+} ions from cell membranes by cationic ACh apparently causes the dramatic ACh-induced increase in membrane permeability in animal cells (4, 9, 14), it may be that the growth-promoting activity of ACh in coleoptile cells is also mediated by calcium

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[2] Abbreviation: ACh: acetylcholine.

displacement from cell walls or cell membranes. Since calcium is known to play an important role in the maintenance of plant cell membrane properties (3, 13, 18), it may be that ACh acts to alter cell membrane permeability in coleoptile cells by displacing calcium from strategic sites within the membrane. If the rate of growth were limited by the rate at which cell wall precursors penetrate the cell membrane, a change in membrane properties that encourages passage of cell wall precursors might lead to a stimulation of cell elongation. This mechanism for the promotion of the rate of cell elongation would still be expected to exhibit a dependence upon metabolic production of cell wall components and hence should be sensitive to cyanide.

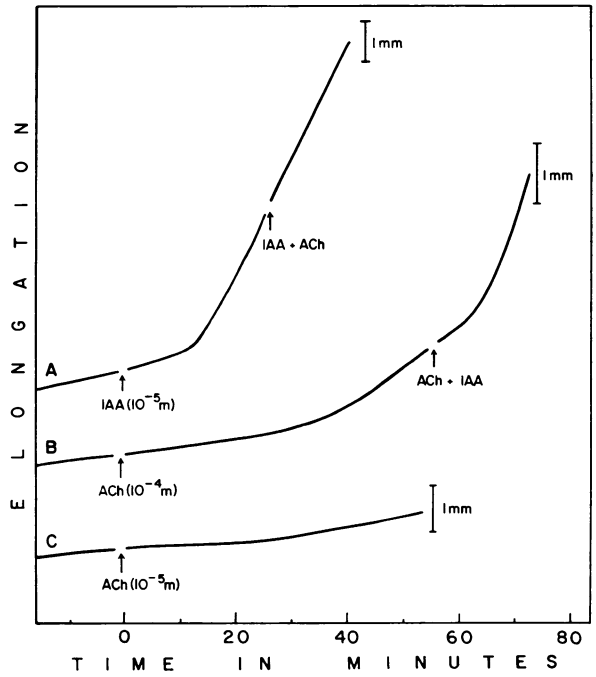

FIG. 1. Time course and magnitude of growth promotion by ACh and IAA. Curve A: growth medium changed from buffer to 10 μM IAA at the first arrow and to 10 μM IAA plus 0.1 mM ACh at the second arrow; curve B: growth medium changed from buffer to 0.1 mM ACh at the first arrow and to 0.1 mM ACh plus 10 μM IAA at the second arrow; curve C: growth medium changed from buffer to 10 μM ACh at the arrow. The vertical bar at the end of each curve in this figure and in subsequent figures represents 1 mm of elongation, for that particular curve, for the entire column of segments.

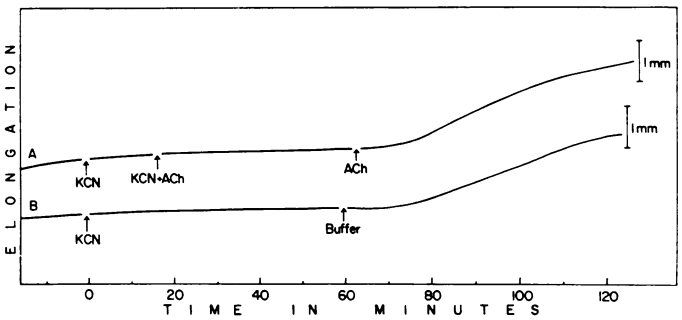

FIG. 2. Cyanide sensitivity of promotion of cell elongation by ACh. Curve A: growth medium changed from buffer to 1 mM KCN at the first arrow and to 1 mM KCN plus 0.1 mM ACh at the second arrow and finally to 0.1 mM ACh alone; curve B: growth medium changed from buffer to 1 mM KCN at the first arrow and back to buffer at the second arrow.

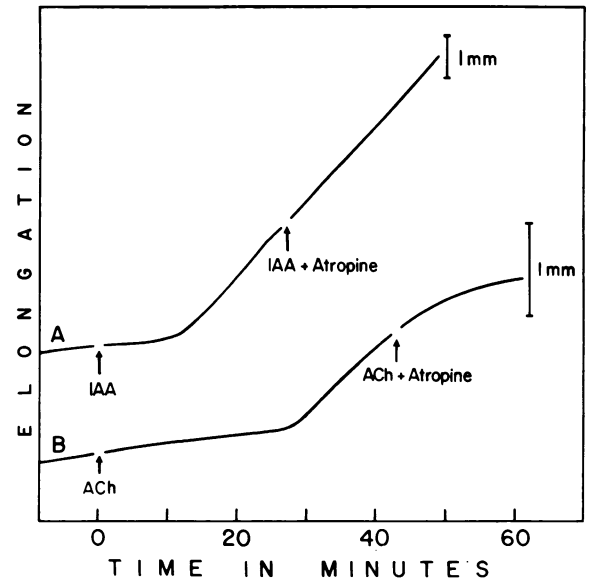

FIG. 3. Selective inhibition of ACh-promoted cell elongation by atropine sulfate. Curve A: growth medium changed from buffer to 10 μM IAA at the first arrow and to 10 μM IAA plus 0.1 mM atropine sulfate at the second arrow; curve B: growth medium changed from buffer to 0.1 mM ACh at the first arrow and to 0.1 mM ACh plus 0.1 mM atropine sulfate at the second arrow.

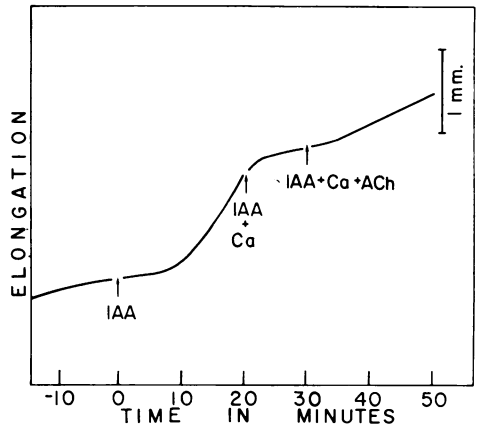

FIG. 4. ACh-mediated reversal of the inhibition of auxin-promoted cell elongation by calcium. Growth medium changed from buffer to 10 μM IAA at the first arrow, to IAA plus 10 mM $CaCl_2$ at the second arrow, and to IAA plus $CaCl_2$ plus 0.1 mM ACh at the third arrow.

Whether the action of ACh in the promotion of cell elongation involves the displacement of calcium or not, a stimulation of calcium displacement and efflux by ACh might be involved in the recently reported phenomenon of ACh-induced binding of root tips to negatively charged glass surfaces (11).

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