The Lack of Effect of Cyclic Adenosine 3':5'-Monophosphate on Avena Coleoptile Growth

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ABSTRACT

The effects of cyclic adenosine 3':5'-monophosphate (cAMP) on the growth of Avena coleoptile segments over 4 to 10 hours were monitored with a position sensing transducer. At pH 6, cAMP (0.1 mM with and without 2.5 mM glucose; or 2 mM alone) or dibutyryl cAMP (0.1 mM) was added at the beginning of the experiment, or after about 1 hour or after about 6 or 7 hours. Under all conditions tested, cAMP compounds had little or no effect on coleoptile segment elongation. Inasmuch as cAMP does not duplicate the rapid and vigorous elongation obtained with 2 μM auxin, the hypothesis that cAMP is a mediator of auxin activity is not supported by experimental evidence in this system. This conclusion is dependent upon the assumption that the cAMP compounds penetrated the tissue.

It is well established that cAMP mediates the activity of a number of mammalian hormones. However, the controversy concerning the possible role of cAMP in plant hormone activity continues. Questions about the existence and activity of cAMP, adenylyl cyclase, cAMP-specific phosphodiesterase, and the possible interaction between cAMP and protein phosphorylation in plant tissue remain unresolved. (1, 17). There are reports which suggest cAMP as a possible mediator for responses to auxin. Some evidence indicates that auxin stimulates rapid increases in endogenous cAMP (13, 26) and promotes the conversion of labeled adenine (2, 24) or ATP (14) to cAMP. Other studies suggest that cAMP may mimic auxin effects in the delay of abscission in debudded Coleus petioles (25), and in the enhancement of wheat (27) and oat (26) coleoptile elongation. In addition it had been found that cAMP has a synergistic effect with auxin in promoting growth in Jerusalem artichoke tissue (15, 16), and with glucose in stimulating Avena coleoptile elongation (11). The transport (9) and distribution (3) pattern of cAMP in certain plant tissues is also similar to that of auxin. On the other hand some investigators report that cAMP alone does not promote growth in dwarf pea stems (18), or in Avena coleoptile segments (4, 11, 12, 27, and M. L. Evans, personal communication).

If cAMP is a mediator of auxin activity, then cAMP should be able to duplicate physiological and biochemical effects of auxin. One well-known response to auxin is the powerful and very rapid promotion of elongation in Avena coleoptiles (7, 21). In the present experiments, growth of 1-cm segments from coleoptiles of 4- to 5-day-old Avena sativa L. cv. Victory was measured with an angular position sensing transducer as described previously (21). Succinate buffer (5 mM, pH 6) was used with appropriate solutions of 2 mM and 0.1 mM cAMP (with and without 2.5 mM glucose), 0.1 mM DB-cAMP (Sigma Chem. Co.), and 2 mM cGMP.

RESULTS AND DISCUSSION

In control runs where coleoptile segments grew in buffer alone, there was some fluctuation in the growth rates between individual trials (Fig. 1). Furthermore, during any one particular trial there occurred two periods of significantly accelerated elongation above the basal growth rate of about 0.03 mm/hr segment. The first, which has been referred to as the “tactile effect,” occurs during the first 45 min and is apparently the result of physical stimulation of the coleoptiles brought about by harvesting and stringing the segments onto the wire in the measuring chamber (5, 7). The second, which has been referred to as the “accelerated endogenous growth effect,” is initiated after about 2 hr and may last from 1 to 2 hr. The cause of this accelerated endogenous growth is unknown, but it seems to be a highly interesting phenomenon (5, 6). To avoid the complicating influence of the tactile effect and the accelerated endogenous growth effect without having to use inhibitors, cAMP was added at one of three different times: (A) at zero time; (B) at or near 1 hr; (C) after 6 or 7 hr (Fig. 2). The IAA curve shown demonstrates the 15- to 20-min latent time and the strong promotion of elongation which occurs when 2 μM IAA is added at time zero or at any subsequent time. The addition of 0.1 mM cAMP did not at any time duplicate the auxin effect. The same results were found when the higher concentrations of 2 mM cAMP (Fig. 3) or cGMP (data not shown) were tested.

Most of the segment growth studies with cAMP reported in the literature have consisted of relatively long term experiments (4, 11, 12, 27) which have involved measurements taken after substantial intervals of time, usually 20 to 24 hr. In some cases (18, 24, 27), no mention is made of pH control during the incubation, and in several others (4, 11, 12) a pH of 5 or 5.2 is mentioned. A pH of 5 is within the range for acid-promoting effects on segment elongation (8, 10, 20, 22). Unbuffered solutions of cAMP tend to be acidic and this...

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2 The concentration of the 0.1 mM cAMP solution was slightly low due to the fact that it was calculated on the basis of the anhydrous molecular weight without allowance for the 1.5 to 2.5 moles of H₂O included.

3 Abbreviations: DB-cAMP: N⁰,O⁰-dibutyryl cyclic 3',5'-adenosine monophosphate; cGMP: cyclic 3',5'-guanosine monophosphate.
might explain the promotive effects found by some workers. Although the use of incubation solutions with an acid pH may not necessarily invalidate the results of a study, it tends to complicate the interpretation of the data. The maintenance of incubation media in the 6 to 7 pH range circumvents this problem.

Hartung (11) found synergistic effects between 0.1 mM cAMP and 2.5 mM glucose at pH 5.2 in the stimulation of coleoptile elongation as determined by measurements made at the end of a 20-hr incubation. We were not able to confirm his reports by continuous measurements made over a 4- to 8-hr period at pH 6 (Fig. 4). Glucose by itself had a strong promotive effect on segment elongation, perhaps because of its capacity as an energy source, but no synergistic effects with cAMP were observed. Similarly, when coleoptile segments pretreated with 0.1 or 1 μM IAA were incubated with both IAA and 2 mM cAMP, no significant synergistic effects were noted (data not shown).

There have been doubts expressed by some workers concerning the capacity of cAMP to penetrate cells. Lack of penetration would result in negative data, similar to those obtained here. It seems likely that cAMP did penetrate the cells of the submerged segments, inasmuch as Gordon et al. (9) found the cut ends of Avena coleoptile segments to take up tritiated cAMP from agar blocks rapidly (within 10 min). DB-cAMP has greater biological activity than cAMP in some animal systems, supposedly because of its greater penetrating capacity (although now questioned, see ref. 23) or resistance
LACK OF cAMP EFFECT IN AVENA

Fig. 3. Elongation of Avena coleoptile segments incubated in 2 mM cAMP and 5 mM succinate buffer, pH 6. Lines A, B, and C represent growth curves where cAMP was added at times indicated by the arrows. The IAA curve represents the growth response to 2 μM IAA added at time zero.

Fig. 4. Elongation of Avena coleoptile segments incubated in 0.1 mM cAMP, 2.5 mM glucose, and 5 mM succinate buffer, pH 6. Lines A, B, and C represent growth curves where cAMP was added at times indicated by the arrows. The IAA curve represents the growth response to 2 μM IAA without glucose added at time zero.

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