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Evidence against mediation of adenosine-3',5'-cyclic monophosphate in the bud-inducing effect of cytokinins in moss protonemata

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Abstract:

Effects of adenosine-3',5'-cyclic monophosphate (cAMP), $N^6,0^2$-dibutyryl adenosine-3',5'-cyclic monophosphate (DBcAMP), caffeine and theophylline on the bud-inducing activity of cytokinin in the protonema of two moss species, *Ceratodon purpureus* and *Funaria hygrometrica* were examined. The substances have been found ineffective as gametophore bud inducers. Some synergism between cytokinin and cAMP or DBcAMP was observed with relation to the buds' growth, but this effect is nonspecific since it can be obtained with 5'-AMP or 5'-GMP as well. The results seem to exclude the possibility of an involvement of cAMP as a second messenger in the mechanism of cytokinin action on morphogenetic processes in moss protonemata.

INTRODUCTION

The mediation of cAMP in the mechanism of action of some animal hormones is known and well documented. Fewer and controversive are data regarding the presence and regulatory function of cAMP in higher plants (Raymond et al. 1973; Bahofen 1973; Becker and Ziegler 1973; Ownby et al. 1973; Hall and Galsky 1973; Wellburn et al. 1973; Amrhein 1974; Bianco and Bulard 1974). Therefore, the problem of cAMP in relation to the action of plant hormones is still unclear and open. Few reports on the possibility of substitution by cAMP of respective hormones in the induction of some processes in plants concerned mainly gibberellins (Pollard 1970 and 1971; Gilbert and Galsky 1972; Hall and Galsky 1972; Kamisaka et al. 1972; Roy et al. 1973; Bianco and Bulard 1974; Goldthwaite

1974) and auxins (Kamisaka et Masuda 1970; Hartung 1972; Kamisaka et al. 1973; Cline et al. 1974). Very little is known about the relationship between cAMP and specific effects of cytokinins. A cytokinin-like activity of N^6,O^2-dibutyryl-cAMP was found in the soybean callus bioassay, this effect, however, was not connected with ribosyl-3, 5-cyclic monophosphate moiety of the molecule but was determined by the N^6-butyryl side chain (Dekhujzen and Overeen 1972). Studying the molecular mechanism of morphogenetic activity of cytokinins it seemed of interest to prove if cAMP and its dibutyryl derivative could replace cytokinins in such a highly specific morphogenetic effect as the induction of gametophore buds in moss protonemata (Bopp 1965; Hahn and Bopp 1968; Szweykowska et al. 1969).

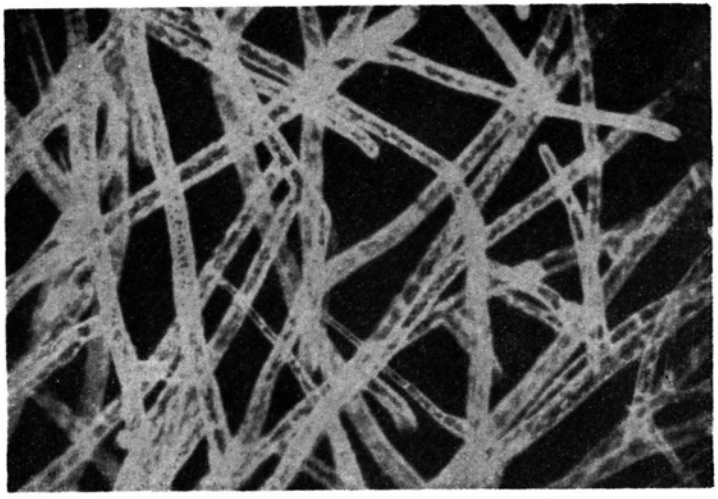
MATERIALS AND METHODS

Protonema cultures of mosses *Ceratodon purpureus* (L. ap. Hedw.) Brid. and *Funaria hygrometrica* (L.) Sibth. were grown from spores. The method of obtaining sterile cultures from spores has been described erlier (Szweykowska and Handszu 1965). In the case of *Ceratodon purpureus* the experiments were carried out using sterile vegetatively propagated clone culture of the protonema. Protonemata of both species were grown in Erlenmayer flasks on cellophane disks (Bopp et al. 1964) placed on the surface of modified mineral medium according to Koffler (1959, see also Szweykowska et al. 1971), solidified with 0.8% agar. Cultures were kept in culture room under continuous white fluorescent light of ca 1000 lux and temp. of ca 25° C.

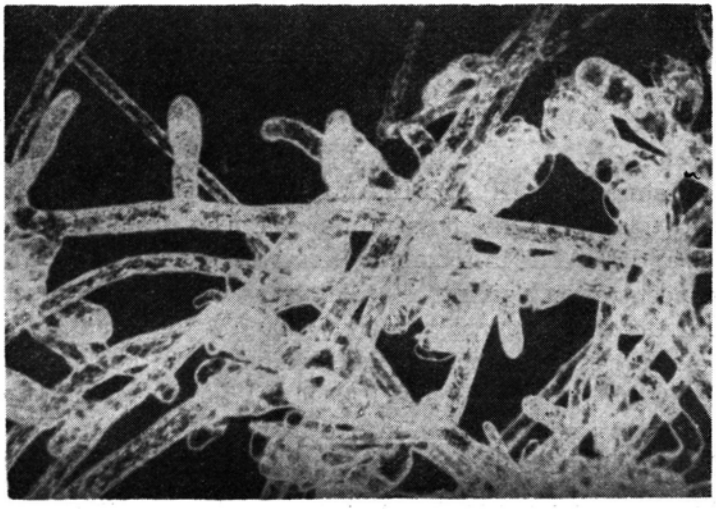
A 30-day-old protonema of *Ceratodon purpureus* and 10-day-old protonema of *Funaria hygrometrica* were transferred to mineral solution (composition same as above) to which the tested substances were included. As basal controls served cultures transferred to mineral medium without any additions. As cytokinin 2iP (6-Δ^2-isopentenylaminopurine) has been used. After 5 days, the bud inducing activity of the test solutions was checked with light microscope. At that time none or only single buds were present in basal controls (Phot. 1.), whereas protonema from cytokinin control produced large number of buds (Phot. 2).

RESULTS AND DISCUSSION

Both cAMP and its derivative, N^6,O^2-dibutyryl adenosine-3',5'-cyclic monophosphate (DBcAMP) — which penetrates cells membranes more easily and is resistant to phosphodiesterase — showed no bud inducing activity in the two investigated moss species. The protonema grown for 5 days on medium with the addition of cAMP or DBcAMP (in concentrations

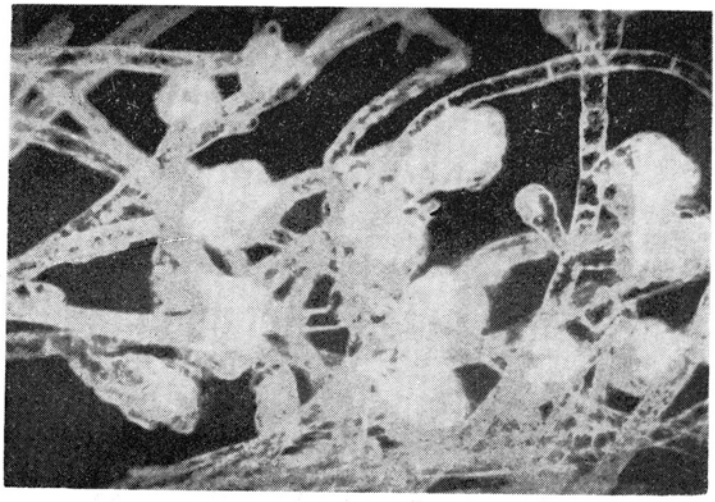


Phot. 1. Basal control. *Funaria hygrometrica*



Phot. 2. Cytokinin control (2iP 0.05 μM). *Funaria hygrometrica*

of 0.1, 1, 10, 100 and 1000 μM, resp.) showed no differences with regard to the control grown on basal medium which was deprived of buds or with sporadically appearing, single gametophore buds only. At the same time large number of buds appeared in media containing 2iP (0.5 μM for *Ceratodon purpureus* and 0.05 μM for *Funaria hygrometrica*). Theophylline and caffeine (inhibitors of cyclic nucleotide phosphodiesterase, inhibiting the breakdown of cAMP and thereby increasing its level in cells, which can mimic or potentiate the action of hormones), used in concentrations of 10, 100, and 1000 μM, resp., were equally inactive as gametophore bud inducers.



Phot. 3. 2iP 0.005 μM + DBcAMP 100 μM. *Funaria hygrometrica*

It has been shown that in some cases the cAMP alone does not mimic the hormone effect in plants, but when used together with hormone — it acts synergistically, potentiating the hormone action (Kamisaka et al. 1973; Kamisaka et Masuda 1971 cit. Gilbert and Galsky 1972). Therefore, it has been decided to check if a synergism exists between cytokinin and cAMP or DBcAMP with respect to the bud-inducing activity of cytokinin in the moss protonema. The protonema was treated with cAMP or DBcAMP in a combination with cytokinin either in a suboptimal concentration, giving a relatively weak bud-inducing effect (0.05 μM for *Ceratodon purpureus* and 0.005 μM for *Funaria hygrometrica*), or in a sub-treshold concentration, ineffective in bud induction (0.005 μM for *Ceratodon purpureus* and 0.0005 μM for *Funaria hygrometrica*). In these conditions, no synergism in bud induction could be found between both cyclic nucleotides and the cytokinin. Protonemata additionally treated with cAMP or DBcAMP did not differ in bud number from those treated with cytokinin only (Table 1). A similar experiment with caffeine and theophylline, applied together with 2iP in concentrations same as above, also gave negative result (Table 1). Some synergism between cytokinin and cAMP or DBcAMP was only observed in relation to buds' growth. Buds from cultures treated with cytokinin in suboptimal concentration and with cAMP or DBcAMP were larger than buds treated with cytokinin only (Phot. 3). This effect, however, appeared to be nonspecific as it could be obtained as well with noncyclic nucleotides, 3'-AMP or 3'--GMP.

Since the bud-induction response to cytokinin is conditioned by age of the protonema (Brandes and Kende 1968; Szweykowska et al. 1969), i.e. by an appropriate stage of physiological maturity, a synthe-

Table 1

Effect of cytokinin (2iP), cAMP, DBcAMP, caffeine and theophylline on gametophore bud induction in moss protonemata

Incubation medium	Result
1. Basal control (mineral solution without any addition)	No buds
2. Cytokinin control (optimal concentration of 2iP: 0.5 μM for *Ceratodon*, 0.05 μM for *Funaria*)	Strong bud-inducing effect
3. cAMP or DBcAMP (0.1, 1, 10, 100 and 1000 μM, resp.)	No buds
4. Caffeine or theophylline (10, 100 and 1000 μM, resp.)	No buds
5. 2iP in suboptimal concentration (0.05 μM for *Ceratodon*, 0.005 μM for *Funaria*)	Buds appear in smaller number than in cytokinin control with optimal conc. of 2iP (see variant No. 2)
6. 2iP in suboptimal concentration +cAMP or DBcAMP (0.1, 1, 10, 100 and 1000 μM, resp.)	The number of buds comparable to that with cytokinin alone (see variant No. 5), but the size of buds is larger.
7. 2iP in suboptimal concentration +caffeine or theophylline (10, 100 and 1000 μM, resp.)	The same effect as with cytokinin alone (see variant No. 5).
8. 2iP in sub-treshold concentration (0.005 μM for *Ceratodon*, 0.0005 μM for *Funaria*)	No buds
9. 2iP in sub-treshold concentration +cAMP or DBcAMP (0.1, 1, 10, 100 and 1000 μM, resp.)	No buds
10. 2iP in sub-treshold concentration + caffeine or theophylline (10, 100 and 1000 μM, resp.	No buds

sis of endogenic agent cooperating with cytokinin has been postulated (K l e i n 1967). In this relation, it was examined if addition of cAMP besides cytokinin could induce bud formation in a juvenile protonema, still insensitive to the cytokinin. A 5-day-old protonema of *Funaria hygrometrica* was treated with an optimal concentration of 2iP together with cAMP (10 and 100 μM). No effect of cAMP could be found — the buds appeared simultaneously in the cytokinin control and in the variant containing additionally cAMP, and not erlier than after ten days of cytokinin

treatment, after the protonema had reached physiological maturity. As previously, a stimulation by cAMP of the buds' growth was noticed.

The results indicate that cAMP and DBcAMP in the tested concentration range are not able to mimic the cytokinin effect in the investigated model system .Considering also the ineffectiveness of caffeine and theophylline, it does not seem possible that the bud-inducing effect of cytokinins in the moss protonema is directly related to an alteration in cAMP concentration. According to criteria applied for animal systems (Pastan and Perlman 1971; Robinson, Butcher and Sutherland cit. Gilbert and Galsky 1972) four conditions need to be satisfied to recognize that a hormone acts through cAMP:

1) The hormone should increase the level of cAMP in the target cells.

2) The hormone should stimulate the activity of adenyl cyclase in extracts from respective cells.

3) Inhibitors of phosphodiesterase should mimic or potentiate the action of the hormone.

4) Exogenous cAMP or its suitable analogue should mimic the physiological effect of the appropriate hormone.

The unfulfillment of the last two criteria gives some basis to exclude the mediation of cAMP in the mechanism of cytokinin-induced differentiation of gametophore buds in mosses, although certitude at this point would only be given by investigations of adenyl cyclase activity and of the endogeneous cAMP level in the protonema cells. Some doubts can also be raised from so far generally accepted interpretation of theophylline and caffeine effects (Hartung 1972; Gilbert and Galsky 1972), as there are data indicating that phosphodiesterases of higher plants are insensitive to methylxanthines (Vandepeute et al. 1973, Amrhein 1974). On the other hand, there is no convincing evidence for a regulatory function of cAMP in higher plants, since even in such a comprehensively investigated system as the barley endosperm the effect of cAMP on α-amylase activity was only sporadically observed (Pollard 1971). Also insufficient and ambiguous are data on the presence and role of enzymes regulating the level of cAMP in higher plants: adenyl cyclase (Sutherland et al. 1962, Alvarez et al. 1974) and phosphodiesterase of cyclic nucleotides (Lin and Varner 1972, Vandepeute et al. 1973). Results obtained recently by Amrhein (1974) make questionable the occurrence of cAMP itself in cells of higher plants. These data provide additional support to our negative conclusion concerning the involvement of cAMP in the mechanism of morphogenetic action of cytokinin in moss protonemata.

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Dowody przeciwko pośrednictwu cyklicznego adenozynu 3',5'-monofosforanu w pączkotwórczym efekcie cytokinin w splątku mchów

Streszczenie

Badano efekt adenozyno 3',5'-cyklicznego monofosforanu (cAMP), N^6,O^2-dwubutyrylo adenozyno-3',5'-cyklicznego monofosforanu (DBcAMP), kofeiny i teofiliny na pączkotwórczą aktywność cytokinin w splątku mchów *Ceratodon purpureus* i *Funaria hygrometrica*. Testowane substancje okazały się nieefektywne jako induktory pączków gametoforowych. Pewien synergizm pomiędzy cytokininą i cAMP lub DBcAMP zaobserwowano w odniesieniu do wzrostu pączków, jednak efekt ten jest niespecyficzny, ponieważ można go uzyskać stosując 5'-AMP lub 5'-GMP.

Uzyskane wyniki wykluczają możliwość udziału cAMP jako pośrednika w mechaniźmie działania cytokinin na procesy morfogenetyczne w splątku mchów.