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Red radiation and choline compounds influence growth and greening of wheat seedlings

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Abstract

The effects of 2-chloroethyltrimethylammonium chloride (CCh), 2-ethyltrimethylammonium chloride (Ch), and acetylcholine chloride (ACh) at concentrations of 1 μM - 5 mM and of red radiation (R) pulse on growth, greening, and formation of the photosynthetic apparatus in etiolated wheat seedlings (*Triticum aestivum* L. cv. Moskovskaya-35) were examined. A short-term application of cholines and R pulse stimulated the first leaf growth and its appearance from coleoptile, and inhibited the coleoptile growth. The effects of cholines were observed during 96 h after the treatment of 4-d-old seedlings and depended on the type and the concentration of cholines. CCh, Ch, and R were also stimulators of greening and increased the photosynthetic activity, whereas ACh did not influence the process of greening. Joint effects of R with cholines on the growth and photomorphogenesis were greater than the individual ones, whereas far-red (FR) radiation decreased the influence of cholines. Thus phytochrome may modify the effects of cholines in the processes of growth and greening.

Additional key words: acetylcholine chloride; 2-chloroethyltrimethylammonium chloride; chlorophyll; cholines; coleoptile length; 2-ethyltrimethylammonium chloride; fluorescence induction kinetics; leaf length; *Triticum aestivum* L.

Introduction

Via the phytochrome system, short-time R controls many primary phases of plant growth and development, in particular the unrolling of first leaf and chlorophyll formation during greening of cereal seedlings (Briggs *et al.* 1988, Kuznetsov *et al.* 1992). Some compounds containing the 2-ethyltrimethylammonium group (cholines) that regulate plant growth act similar to R; moreover, they may modify and

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Abbreviations: ACh - acetylcholine chloride; CCh - 2-chloroethyltrimethylammonium chloride; Ch - 2-ethyltrimethylammonium chloride; FR - far-red radiation; PS - photosystem; R - red radiation.

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significantly strengthen phytochrome-controlled photomorphogenic responses. For example, some phytochrome-controlled morphogenic responses involve acetylcholine, the main component of the cholinergic system of higher plants (Jaffe 1970, Gressel *et al.* 1971, Kopcewicz *et al.* 1977, Tretyn *et al.* 1990, Tretyn and Kendrick 1990, 1991). We demonstrated that the plant growth retardant CCh (Cathey 1964, Deeva 1980), when applied exogenously to seeds, acted as R and stimulated the growth of the second leaf and chlorophyll formation during the greening of etiolated wheat seedlings (Vasilenko *et al.* 1991, Kuznetsov *et al.* 1992). Futhermore, CCh modified the phytochrome-controlled responses of the unrolling of the first leaf and chlorophyll formation. Its application eliminated the reversibility of R by the following FR irradiation.

However, the question remains if the phytochrome-controlled responses interacted with CCh or with its products, since the CCh treatment was applied to seeds and the R was applied to 5-d-old seedlings. Because of the structure-relationship (the same 2-ethyltrimethylammonium group) between CCh, Ch, and ACh, we compared their effects on the photomorphogenetic responses during greening of etiolated wheat seedlings. We also studied the interaction of simultaneous R and choline treatments on the growth, greening, and formation of photosynthetic apparatus.

Materials and methods

Wheat seedlings (*Triticum aestivum* L. cv. Moskovskaya-35) were grown in petri dishes in the dark at 23 °C. 4-d-old etiolated seedlings were treated by root application with 1 μM-5 mM water solutions of Ch, CCh, or ACh (*Serva*, Germany) for 1.5 h, and then irradiated by R (λ_{max}= 660 nm; $\Delta\lambda_{1/2}$ = 8 nm; I = 2 W m^{-2}) or FR (λ_{max} = 730 nm; $\Delta\lambda_{1/2}$ = 9 nm; I = 3 W m^{-2}) for 7.5 min depending on the treatment. In comparative experiments cholines were applied after R (FR) irradiation. The two types of stimuli (cholines and radiation) were given sequentially in saturating amounts. To test the R/FR photoreversibility, short-term FR was switched once after R (R+FR). After irradiation the seedlings were kept for 24 h in the dark, then the growth parameters were measured. Growth measurements were also repeated after 48, 72, and 96 h. The greening of 4-d-old seedlings was studied at treatment with choline compounds 1.5 h before or after R (FR) irradiation. After 24 h, the seedlings were exposed to continuous "white light" from luminescence tubes (I = 45 W m^{-2}). The content of chlorophyll (a+b) was determined as described by Lichtenthaler and Wellburn (1983) using spectrophotometer *Specord M-40* (Germany). Curves of delayed chlorophyll fluorescence of greening leaves were registered by a phosphoroscope set-up using the two-beam method (Krause and Weis 1991). Type II broken chloroplasts were isolated after 24 h of greening of wheat seedlings by a modified method of West and Wiskich (1968). Five to seven independent repetitions with 60-100 plants per experiment were used for the determinations of growth parameters, chlorophyll content, and fresh masses of leaves. The *t*-test was used to find the significant differences.

Results

The R treatment as well as the treatments with cholines accelerated the first leaf elongation and its appearance from coleoptile; they also inhibited the coleoptile elongation. These effects were reversible by short-term FR (Fig. 1). The leaf length increased and coleoptile length decreased in correspondence with increasing choline concentration (Fig. 2): maximum effects were at 0.01-0.10 mM for Ch and at 0.1 mM for CCh, but no obvious maximum was found for ACh. The effects of Ch were slightly greater than those of CCh and significantly greater than those of ACh that were significant only at high concentration. Maximum stimulation of the first leaf growth by Ch and CCh was observed when the 4-d-old seedlings were treated, and a minimum one in case of treatment of caryopses (values not shown). In contrast to the individual application of cholines, their combinations with R were more efficient at all applied concentrations, indicating in some cases synergism. In a similar manner, the pretreatment with cholines increased the growth effects of R+FR combinations (for example, R+FR < Ch+R+FR < Ch+R+FR+R; Fig. 1). The treatment with cholines for 1.5 h after R (FR) irradiation led to similar results.

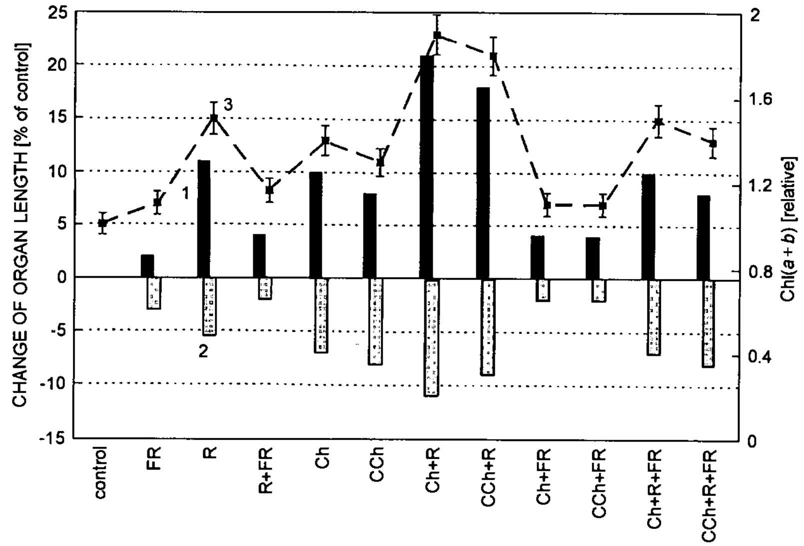

Fig. 1. Cooperative and individual effects of red radiation (R), far-red radiation (FR), and choline compounds 2-ethyltrimethylammonium chloride (Ch) and 2-chloroethyltrimethylammonium chloride (CCh) on leaf (*1*) and coleoptile (*2*) growth and chlorophyll accumulation (*3*). The 4-d-old seedlings were treated with 0.1 mM Ch or CCh during 1.5 h and then R or FR pulse was given. In experiments with green seedlings the 5-d-old seedlings were exposed to a continuous "white light" (I = 40 W m^{-2}) and after 24 h of greening the concentration of chlorophyll [Chl (*a*+*b*)] was determined [per 1 g(fresh biomass)]. The results are the average of five independent experiments.

Ch, CCh, and R treatment increased the chlorophyll content in 5-d-old greening seedlings (Fig. 1) and partly eliminated the lag-phase in chlorophyll accumulation at concentrations of 1 μM-5 mM (values not shown). We did not find any similar effect of ACh. The maximum effects of cholines and R or their combinations were observed at the initial stage of greening (6-12 h). In combination with R, the stimulatory influence of Ch or CCh was much larger than the individual effects. FR itself did not act, although it partly eliminated the stimulatory effects of cholines (Fig. 1).

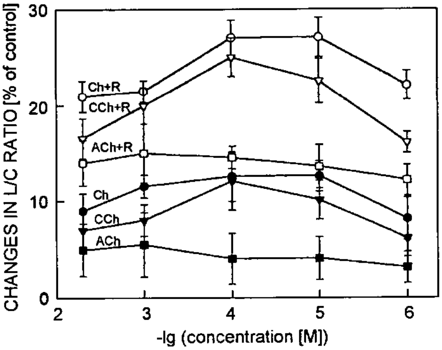

Fig. 2. Dependence of the L/C (leaf length/ coleoptile length) ratio on the concentration of applied choline compounds. The scheme of treatments, irradiations, and abbreviations is the same as in Fig. 1 (*n* = 4). The L/C ratio of control was taken as 100 %.

Curves of variable chlorophyll fluorescence showed that the F_v/F_m ratio increased after the treatment of 4-d-old etiolated seedlings with Ch or CCh (0.01-0.10 mM) during 7 and 24 h of greening by *ca.* 10-20 % compared to control (Table 1). These effects were a little larger than those induced by R. No effect of ACh (0.01-0.10 mM) was found. An increase in the slope of the delayed chlorophyll fluorescence curves was also observed after R and choline treatments (Fig. 3). The application of Ch or CCh (1-100 μM) did not induce changes in the fluorescence ratio as measured in suspensions of chloroplasts isolated from greening leaves of wheat seedlings exposed to "white light" for 24 h.

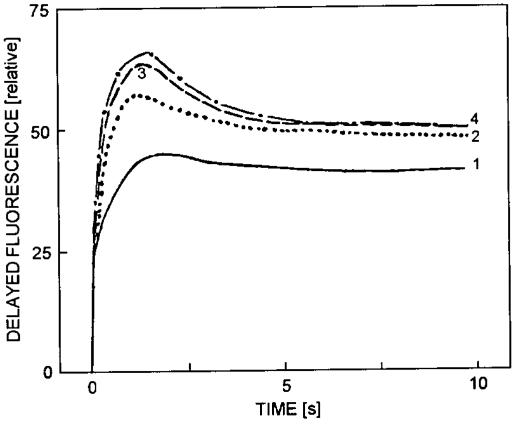

Fig. 3. Delayed fluorescence induction kinetics after 6 h of greening of 5-d-old etiolated seedlings in a continuous "white light" (I = 40 W m^{-2}). *1* - control, *2* - red radiation (R), *3* - 2-ethyltrimethylammonium chloride (Ch), *4* - Ch+R. The 4-d-old seedlings were treated with 0.1 mM Ch during 1.5 h and then a R pulse was given.

Table I. Changes in the F_v/F_m ratio depending on the treatment and the time of greening. 4-d-old seedlings were treated with 0.1 mM 2-ethyltrimethylammonium chloride (Ch), 2-chloroethyltrimethylammonium chloride (CCh), or acetylcholine chloride (ACh) during 1.5 h and then a red (R) or far-red (FR) pulse was given. The values are means ± SE ($n = 4$); *significant ($p < 0.05$) differences between control and treatments.

	Time [h] 7	 24
Control	0.60 (0.020)	0.68 (0.015)
R	0.68 (0.015)*	0.73 (0.020)
Ch	0.69 (0.015)*	0.74 (0.010)*
Ch+R	0.70 (0.020)*	0.75 (0.010)*
CCh	0 68 (0.010)*	0.73 (0.020)
CCh+R	0.71 (0.020)*	0.76 (0.015)*
ACh	0.61 (0.020)	0.69 (0.020)

Discussion

Earlier photomimetic effects in phytochrome-dependent processes were indicated only for ACh, the main component of cholinergic system in higher plants, found in many plant species (Miura and Shih 1984, Hartmann and Gupta 1989, Tretyn and Kendrick 1991). In our experiments relatively weak growth effects of ACh were considerably strengthened after additional R treatment [ACh (~ control) < ACh+R]. These effects, *i.e.*, the stimulation of the first leaf growth and the inhibition of coleoptile, are also typical for other cholines, Ch and CCh (Fig. 1), the structure analogues of ACh. Growth responses were maximal for Ch and minimal for ACh; they were observed at least during several days after application of all tested concentrations of CCh and Ch and at high concentrations of ACh. According to a model of Hartmann and Gupta (1989), minimal effect of exogenic ACh may be due to its fast hydrolysis by the enzyme acetylcholine esterase. In our experiments the cholines stimulated growth and similarly to short-term R promoted the first leaf elongation, the first leaf emergence, and the coleoptile growth inhibition. We did not check the effects for a long time after application of cholines but we indicated the inhibitory action of 5×10^{-3} M CCh, when applied exogenously to seeds, on the first leaf growth in etiolated wheat seedlings by the sixth day (values not shown).

The growth and development of the first leaf and coleoptile in etiolated wheat seedlings are phytochrome-controlled processes (Tretyn and Kendrick 1990, Vasilenko *et al.* 1991). Our results (Fig. 1) support this conclusion and show a close similarity between actions of R and cholines in regulation of the processes. The mutual strengthening of the effects of R and choline compounds [ACh < CCh < Ch (= R) < ACh+R < CCh+R < Ch+R; Figs. 1 and 2] seems to be the most important phenomenon. Another interesting phenomenon, similar to the R/FR reversibility (Fig. 1), is the partial elimination of the effects of Ch and CCh on leaf and coleoptile by FR. This result confirms our conclusions about a close connection of the phytochrome system with the action of cholines. However, a direct action of cholines on the level of active form of phytochrome (P_{FR}) does not seem to take place since the growth responses were identical in variants Ch+FR and FR+Ch.

One of the early stages of plant photomorphogenesis is deetiolation, stimulated by R (Kasemir 1983, Briggs *et al.* 1988). Little is known of the action of cholines on photomorphogenic responses such as growth, photosynthetic apparatus formation, and chlorophyll accumulation during the greening of etiolated seedlings (Kuznetsov *et al.* 1992). The effects of Ch and ACh on photosynthetic activity and chlorophyll content in green plants were studied by Roshchina and Mukhin (1985), Hyeon *et al.* (1987), Tretyn and Kendrick (1991), *etc.* Increased CO_2 fixation and O_2 evolution was found in wheat protoplasts treated with Ch (Hyeon *et al.* 1987). Nevertheless, many choline analogues, including CCh, the chlorinated derivative of choline, were ineffective in acceleration of photosynthesis of protoplasts. On the other hand, CCh treatment raised chlorophyll content and photosynthetic activity in leaves of some plant species (Tezuka *et al.* 1989, Kuznetsov *et al.* 1992).

We found that not only R but also 1 μM-5 mM Ch and CCh (and not ACh) can stimulate chlorophyll (*a*+*b*) accumulation (Fig. 1) and significantly eliminate its lag-phase in wheat seedlings. The maximum effects of cholines and R were found at the initial phase of greening, although they occurred also in the second leaf after one week of greening. The ability of Ch and CCh at low concentrations and root application to eliminate the delay in chlorophyll formation coincides with the action of R on the process of greening (Kasemir 1983, Briggs *et al.* 1988, Kuznetsov *et al.* 1992).

In combination with R the stimulatory influence of Ch or CCh on the process of greening was much larger than the individual effects (for example, Ch < Ch+R = R+Ch), the joint action being noticeable over prolonged period. ACh was appreciably effective only in combination with R (ACh < ACh+R). Similar to growth effect, the R/FR neutralizing effect on chlorophyll formation was somewhat diminished after the preliminary treatment with Ch and CCh (R+FR < Ch+R+FR). The photoreversibility typical for inductive phytochrome-dependent processes did not change too much in our experiments (for example, R/R+FR ~ Ch+R/Ch+R+FR, Fig. 1). Earlier we found another interaction: disappearance of R/FR photoreversibility, promoted by previous pretreatment of caryopses with CCh (Vasilenko *et al.* 1991, Kuznetsov *et al.* 1992). It may be the result of interaction between effects of R and CCh hydrolysis products that could be formed for 5 d after the treatment of seeds with CCh.

FR itself somewhat decreased the individual effects of Ch and CCh on the processes of growth and greening. This supports the idea about the interaction between effects of cholines and the phytochrome system in wheat seedlings.

Our results on photochemical activity of leaves proved that, similar to R, Ch and CCh could enhance the efficiency of photosystem 2 and, since the concentration of applied cholines (0.01-0.1 mM) was low, the effect was specific to cholines. Furthermore, Ch and CCh as well as R increased the slope of the delayed Chl fluorescence curves (Fig. 3), which characterizes the strengthening of the Calvin cycle activity (Krause and Weis 1991). Joint application of R and cholines led to a notable strengthening of their individual effects, that was especially evident at the initial stage of greening. Thus, cholines (excluding ACh) both at root application and at application to seeds (Kuznetsov *et al.* 1992) accelerate the formation of the photosynthetic apparatus during greening similarly to R.

Cholines affected the photosynthetic efficiency (the F_v/F_m) in whole leaves, whereas Ch and CCh had no effect on this ratio when measured in suspensions of isolated wheat chloroplasts. Significant changes in photochemical activity of chloroplasts with added ACh have not been found by Roshchina and Mukhin (1985). Probably, there is no direct influence of cholines on the activity of PS2, which can be evaluated *via* variable fluorescence (Krause and Weis 1991). On other hand, Hyeon *et al.* (1987) reported that Ch increased the photochemical activity of wheat protoplasts, possibly stimulating the activity of ribulose-1,5-bisphosphate carboxylase. Tezuka *et al.* (1989) found increased carbon assimilation in leaves of hollyhock plants after application of CCh: this effect may be due to raising both the CO_2 uptake and chlorophyll accumulation. In addition to this, choline and short-term R treatments can enhance photosynthetic efficiency and chlorophyll synthesis indirectly by changing cell membrane permeability (Fabijan *et al.* 1981, Kendrick and Bossen 1987) that influences the activity of appropriate enzymes.

We conclude that CCh and Ch (1 μM-5 mM) at root application stimulate the growth of the first leaf, Chl accumulation, and photosynthetic apparatus formation but ACh is effective in growth processes only if applied at high concentration. Thus, these responses depend on chemical structure of the compounds and are modifed by short-term R and FR given at saturating amounts.

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