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Original scientific paper

REGULATION OF CHLOROPLAST REACTIONS BY SECONDARY METABOLITES ACETYLCHOLINE AND BIOGENIC AMINES

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Isolated pea chloroplasts were treated with secondary metabolites acetylcholine, dopamine, adrenaline, noradrenaline, serotonin known as animal neurotransmitters or mediators and found in plant cell as well. There were no effects of all compounds on the NADP-photoreduction. Low concentrations ($< 10^{-5}$ M) of these, except serotonin, stimulated noncyclic photophosphorylation in thylakoids. Acetylcholine induced Na^+ , K^+ output from intact chloroplasts, but had no effect on Ca^{2+} , Mg^{2+} efflux. Dopamine, adrenaline, noradrenaline and serotonin had an opposite effect. A possible regulatory and mediatory role of the substances in chloroplasts and in cell is discussed.

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Introduction

According to Frey-Wyssling hypothesis, membranes of chloroplasts can originate from plasmalemma which is sensitive to external chemicals. There is an evolutionary likeness between both types of membranes as chemical sensors. Plasmalemma can receive signals of environment directly from external media while chloroplast — indirectly from internal cell media possibly by means of mediators as has been observed between animal cells in synapse (Roshchina 1989). The role of neuromediators are characteristic of cholinergic acetylcholine and biogenic amines dopamine, adrenaline, noradrenaline and serotonin, forming cholinergic, dopaminergic, adrenergic, serotonergic systems of regulation. Such substances as acetylcholine (Fluch and Jaffe 1976), dopamine, noradrenaline (Udenfriend et al. 1959), adrenaline (Askar et al. 1972) serotonin (Regula 1986) were found in plant cells as well. Acetylcholine is a part of the cholinergic regulation system in animals, including cholinergic receptor, enzyme of the acetylcholine synthesis (choline acetyltransferase) and hydrolysis (cholinesterase). Acetylcholine, choline acetyltransferase and cholinesterase were found in plants (Fluck and Jaffe 1976, Hartmann and Gupta 1989). Acetylcholine, cholinesterase, adrenaline and noradrenaline were also observed in isolated intact chloroplasts (Roshchina 1988, 1989).

We do not exactly know the function of the substances in plastids. Since acetylcholine and biogenic amines were found in multicellular plants and animals without nervous system, their intracellular role is assumed (Buznikov 1987) to be regulators of development or (and) energy and metabolism. It is possible that they are involved in signalling between plasmatic membranes and organelles and between organelles (Roshchina 1989). Intracellular mediation and regulation by them could be a more ancient mechanism than the known role of intercellular mediators in synapse between cells of animals with nervous system (Buznikov 1987). This possibility needs experimental and theoretical analysis. A model of the study could be isolated chloroplasts as a test for the action of the exogenous acetylcholine and biogenic amines.

The aim of the paper was to analyze the regulatory function of the substances in chloroplasts.

Methods

Subjects of the study were chloroplasts isolated from the leaves of 12-day-old seedlings of pea (*Pisum sativum* L., cv. *Prevoskhodnii*), bean (*Phaseolus aureus* L.), stinging nettle (*Urtica dioica* L.). Intact type A chloroplasts were isolated according to Robinson et al. (1979) and type B chloroplasts according to Roshchina and Mukhin (1985). The NADP⁺-photoreduction and coupled photophosphorylation were measured in type B chloroplasts as described earlier (Roshchina and Mukhin 1985), and the Na⁺, K⁺, Ca²⁺, Mg²⁺ output from intact plastids with ion-selective electrodes (Roshchina 1987). Isolation and purification of plastocyanin, ferredoxin, ferredoxin-NADP-reductase from pea leaves and cytochrome f from *Chlorella* cells were done as described earlier (Roshchina 1989). Acetylcholine in leaves and intact chloroplasts was determined by paper chromatography (Roshchina 1989). Cholinesterase activity was measured according to Ellman et al. (1961).

Results

Table 1 shows sensitivity of intact chloroplasts and type B chloroplasts without envelope to exogenous acetylcholine and biogenic amines measured as concentration affecting ion permeability and photochemical activity. Low concentration of acetylcholine stimulated Na^+ , K^+ efflux from intact chloroplasts, but there was no effect on Ca^{2+} , Mg^{2+} output through envelope. Noradrenaline, adrenaline and serotonin had an opposite action. Response reactions of thylakoids to these substances were estimated as changes in photochemical activity. The compounds studied were not sensitive to rates of the NADP^+ -photoreduction. Low concentrations of exogenous acetylcholine, noradrenaline, adrenaline stimulated non-cyclic photophosphorylation ~2-fold, but dopamine did so only to 30% of control (R o s h c h i n a 1989). Serotonin did not act on this reaction. It could be supposed that due to phenolic or indolic groups, biogenic amines are capable of redox reactions. High concentrations of catecholamines interacted with individual electron carriers (Table 2), reducing cytochrome f and plastocyanin. Serotonin had no such effect. Thus catecholamines can take part in redox reactions with metal-containing proteins of the electron transport chain.

Table 1. S_{50} (M), concentrations of acetylcholine and biogenic amines stimulated the pea chloroplasts reactions on 50% of control (0, no effect; —, no data)

| Substances | Intact chloroplasts | | | | Thylakoids | |
|---------------|---------------------|--------------|------------------|--------------------|--|--------------------|
| | Ion efflux | | | Mg^{2+} | NADP^+ photo- reduction | ATP synthesis |
| | Na^+ | K^+ | Ca^{2+} | | | |
| Acetylcholine | 10^{-7} | 10^{-7} | 0 | 0 | 0 | 5×10^{-9} |
| Dopamine | — | — | — | — | 0 | 5×10^{-9} |
| Noradrenaline | 0 | 0 | 10^{-9} | 10^{-9} | 0 | 7×10^{-9} |
| Adrenaline | 0 | 0 | 10^{-9} | 10^{-9} | 0 | 10^{-8} |
| Serotonin | 0 | 0 | 10^{-9} | 2×10^{-9} | 0 | 0 |

Table 2. Redox interaction of high concentrations (10^{-6} — 10^{-3} M) of biogenic amines with isolated electron carriers (0, no effect)

| Substances | Cytochrome f | Plastocyanin | Ferredoxin | Ferredoxin- NADP- reductase |
|---------------|--------------|--------------|------------|-----------------------------------|
| Dopamine | reduced | reduced | 0 | 0 |
| Noradrenaline | reduced | reduced | 0 | 0 |
| Adrenaline | reduced | reduced | 0 | 0 |
| Serotonin | 0 | 0 | 0 | 0 |

Table 3. The acetylcholine content and the cholinesterase activity in isolated intact chloroplasts of higher plants (traces, SE 15%)

| Plant | Acetylcholine $\times 10^{-6}$ (mol kg ⁻¹ of fresh leaf mass) | Hydrolysis of acetylthiocholine $\times 10^{-6}$ (mol s ⁻¹ kg ⁻¹ of fresh leaf mass) |
|----------------------------|--|---|
| <i>Pisum sativum</i> L. | 0.2 | 39 |
| <i>Phaseolus aureus</i> L. | 49 | 1.1 |
| <i>Urtica dioica</i> L. | traces | 29 |

The phenomena observed can take place in vivo and be dependent on the rates of synthesis and katabolism of acetylcholine and biogenic amines. For example, the acetylcholine content and cholinesterase activity were compared for some species (Table 3). Both parameters were estimated by the amount of chloroplasts isolated from 1 kg of fresh leaf mass. The lowest accumulation of choline ester in chloroplasts of pea and sting nettle correlated with the highest activity of the enzyme hydrolyzing it. The opposite correlation was observed for bean plastids.

Discussion

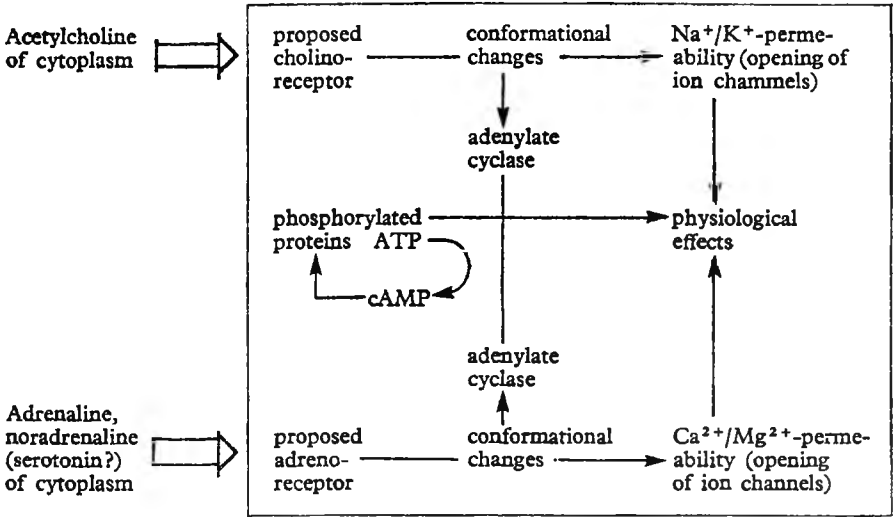
Our results show that in low concentrations acetylcholine and catecholamines can be regulators of ion permeability and photophosphorylation in chloroplasts. As for serotonin, it seems to regulate only Ca²⁺ and Mg²⁺ permeability but to be non-effective on photophosphorylation. These reactions can be estimated as replies of chloroplasts to the intracellular chemical signalling by acetylcholine and biogenic amines. This possibility becomes more real because acetylcholine and catecholamines were found not only in cytoplasm of plant cell, but also in chloroplasts. Other arguments should be sought in evolution.

Photosynthetic organelle chloroplasts were an independent organism in ancient times which then during evolution was possibly included into eukaryotic cell by endocytosis. Symbiont preserved the envelope formed by a double membrane layer and thylakoids as internal structures. The envelope can receive a chemical signal from cytoplasm and thylakoids from stroma as it happened with plasmalemma.

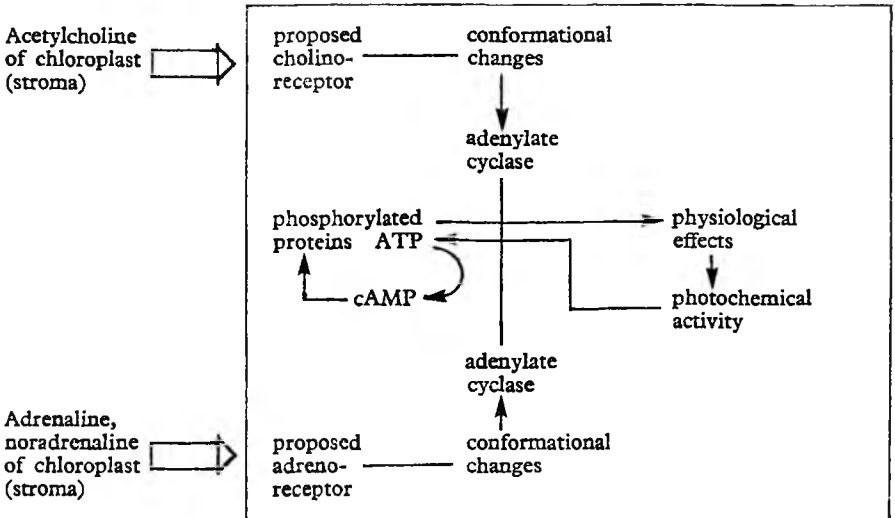
Molecular targets of the acetylcholine and catecholamines action in chloroplasts can be specific receptors, conformational changes of which in response to the binding of the active substance induce shifts in rates of photophosphorylation and Na⁺, K⁺, Ca²⁺, Mg²⁺ output. The reactions using agonists (muscarine and arecoline) and antagonists (atropine and d-tubocurarine) of acetylcholine were shown in earlier papers (R o s h c h i n a 1987, 1989). The functional analog of cholinoreceptor which binds with chemical agents and is excited (or broken) by them was supposed to regulate the opening of ion channel. The presence of adrenoreceptors in chloroplasts is proposed as well, based on the sensitivity of plastids to catecholamines. In animals cholino- and adrenoreceptors regulate not only ion permeability but also the triggering of adenylate cyclase activity.

Scheme 1

The envelope membrane



The thylakoid membrane



This enzyme catalyzes the synthesis of secondary messenger cyclic AMP (cAMP) (Brown and Newton 1981). cAMP phosphorylates membrane proteins. Phosphorylation of proteins is a common regulatory mechanism in physiological processes. Similar ways of regulation are proposed for chloroplasts (Scheme 1) based on the presence of adenylate cyclase

(Brown and Newton 1981) and protein kinase (Soll 1988) in plastids, the protein phosphorylation in chloroplasts (Suss 1981), stimulation of ATP synthesis in thylakoids by acetylcholine and biogenic amines (ATP can be a substrate for cAMP synthesis), changes in ion permeability of envelope of plastids by acetylcholine and catecholamines.

Intracellular functions of the substances are more ancient than their specialized role as intercellular neuromediators (Buznikov 1987). Possible reasons for this conclusion are common distribution of acetylcholine and catecholamines in both kingdoms (animals and plants), the presence of the substances, their receptors and cholinesterase in unicellular and multicellular organisms, common membrane reactions on biomediators (changes in ion permeability and activation of adenylate cyclase). Acetylcholine and catecholamines can play the role of regulators of energetic and transport processes as well as of chemical signals or mediators in the information transmission from cytoplasm to plastids or other organelles.

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SAŽETAK

REGULACIJA REAKCIJA KOROPLASTA SEKUNDARNIM METABOLITIMA
ACETILHOLINOM I BIOGENIM AMINIMA*Viktoriya V. Roshchina*

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Izolirani kloroplasti tretirani su sekundarnim metabolitima acetilholinom, dopaminom, adrenalinom, noradrenalinom i serotoninom, koji su prisutni u biljnim stanicama. U životinja također dolaze ovi spojevi i imaju ulogu neurotransmitera ili medijatora. Svi osim serotonina u niskim koncentracijama ($< 10^{-5}$ M) potiču necikličku fotofosforilaciju u tilakoidama. Acetilholin pospješuje izlaženje K^+ i Na^+ ijona iz intaktnih kloroplasta, ali ne utječe tako i na Ca^{2+} i Mg^{2+} . Dopamin, adrenalin, noradrenalin i serotonin imaju suprotan učinak.

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