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Neurotransmitter Acetylcholine Comes to the Plant Rescue

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Abstract

Acetylcholine is the most common and functional neurotransmitter in humans, produced at the synaptic junctions during the relay of nerve impulse along neurons. This molecule is also reported across several taxonomic groups throughout the plant kingdom, along with the presence of the enzymes, choline acetyltransferase and acetylcholinesterase involved in acetylcholine metabolism, similar to human systems. The maximum level of acetylcholine synthesis occurs in the youngest growing parts and leaves. It performs a host of vital activities in plants ranging from seed germination and plant growth, mimicking the action of red light, influencing leaf movement and membrane permeability to ions, and modifying some enzyme activities and metabolic processes of plants. Acetylcholine has also been observed to exert an influential role in conferring protection to plants against certain environmental constraints and abiotic disturbances. However, due to lack of adequate studies, there is still a wide gap in our understanding of the multiple roles of acetylcholine in plant morphogenesis and overall physiology as well as its ameliorative effect in rescuing the plants from a wide range of stresses and adverse situations. The present mini-review highlights the overall perspectives in this field of research that has advanced so far.

Keywords: Acetylcholine; Plant defense; Physiological regulations; Choline acetyltransferase; Acetylcholinesterase

Introduction

Acetylcholine, an ester of choline and acetic acid, is the most exemplary and well-known neurotransmitter that was first isolated in 1914. It is normally synthesized in animals at the synaptic junctions, nerves and motor end plate of vertebrate muscles from choline and acetyl-coenzyme A (acetyl-CoA) through the enzyme choline acetyltransferase. The rate limiting step of acetylcholine synthesis constitutes choline uptake and transport from the extracellular fluid to the nerve terminal through a sodium-dependent carrier [1]. Following synthesis, acetylcholine is transported into the synaptic vesicles (almost 50,000 molecules per vesicle). Upon perception of a nerve impulse at the nerve ending, the vesicle-stored acetylcholine is released, being initiated by Ca^{2+} influx through voltage-operated N- or P-type calcium channels. The increased intracellular Ca^{2+} binds to a vesicle-associated protein (synaptotagmin) which favors association of a second vesicle protein (synaptobrevin) with one or more proteins in the plasma membrane of the nerve terminal. This vesicle-docking process is immediately followed by the fusion between vesicle membrane and plasma membrane. The released acetylcholine at the synaptic cleft following exocytosis associate with post- and pre-junctional receptors causing depolarization and later subjected to rapid hydrolysis into choline and acetate by acetylcholinesterase enzyme, whereas the empty vesicle is recaptured by endocytosis for reuse. The choline formed is recycled back to the nerve terminal for another round of neurotransmitter synthesis and neuronal impulse signaling [2]. Cholinergic system in animals thus plays a vital role in the transmission of information perceived by the receptors in the form of electrical impulse along the neurons. It comprise of four elements, *viz*., acetylcholinesterase, choline acetyltransferase, acetylcholine receptor and acetylcholine [3].

Till date, the biological function of acetylcholine is mostly focused on its neurotransmitter function in animal systems including humans. However, acetylcholine has also been reported from the cells of several non-nervous tissues in animals, including epithelial cells, smooth muscle cells, mast cells, lymphocytes and alveolar macrophages, so as to suggest a non-nervous function of it. In humans, acetylcholine and/or the synthesizing enzyme, choline acetyltransferase, have been demonstrated in epithelial (airways, alimentary tract, urogenital tract, epidermis), mesothelial (pleura, pericardium), endothelial, muscle and immune cells (granulocytes, lymphocytes, macrophages, mast cells). This widespread distribution of acetylcholine highlights the broader importance of this neurotransmitter in diverse cellular and signaling pathways.

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Acetylcholine Pathway in Plants

Acetylcholine has been detected in bacteria, algae, protozoa, tubellariae, fungi, moss, *Equisetum robustum* and primitive plants, tracing back to early phylogenetic appearance of this molecule in evolution. Regulation of diverse functions like gene expression, immune functions, differentiation, proliferation, cell-cell contact, cytoskeletal organization, ciliary activity, locomotion, migration, absorption, secretion, etc., by non-neuronal acetylcholine in animal cells prompted the plant scientists to explore acetylcholine functions in higher plants. Although plants lack a well-elaborate nervous system, both acetylcholine and acetylcholine-hydrolyzing activity have been widely recognized in the plant kingdom. *Urtica dioica* was one of the early plant members where the function of acetylcholine in terms of regulation of water resorption and photosynthesis was ascertained. Riov and Jaffe (1973) [4] purified and characterized a cholinesterase enzyme from mungbean roots. Sagane et al., (2005) [5] have purified and characterized the molecular composition of maize acetylcholinesterase enzyme and also performed the nucleotide sequence coding for the corresponding cDNA. Smallman and Maneckjee (1981) [6] confirmed the presence of choline acetyltransferase in nettles (*Urtica dioica*), known to possess high levels of acetylcholine, as well as from other plants like peas (*Pisum sativum*), spinach (*Spinacia oleracea*), sunflower (*Helianthus annuus*) and blue-green algae by using a Sepharose-CoASH affinity column. Miura and Shih (1984) [7] quantified acetylcholine by Gas Chromatography/Mass Spectrometry (GC-MS) from several plant species, regardless of the organ (leaves, stems, or roots) or developmental stage (seedlings, mature plants, or seeds). The acetylcholine level was directly correlated with the level of its precursor, *viz*., choline. They also showed that acetylcholine serves as intracellular messenger whose synthesis is promoted by red light, while far-red light or darkness favored the reverse effect. Acetylcholine was quantified in *Pharbitis nil* seedlings through pyrolysis-gas chromatography in all the organs like seeds, cotyledons, shoot apex, leaves, shoot and root, the highest level being recorded in the youngest growing parts [8]. Choline acetyltransferase is responsible for synthesis of acetylcholine from its precursors, choline and acetyl coenzyme A, particularly in young leaves. Acetylcholine in plant cells acts *via* regulation of membrane permeability to protons, potassium ions, sodium ions and calcium. Although there is no direct evidence of the presence of acetylcholine receptors in plants, the application of specific agonists such as muscarin and nicotine, and antagonists such as atropine and tubocurarine indirectly suggests the possible existence of the two main receptor types (muscarinic and nicotinic), which probably serves as acetylcholine receptor, competitively bound by selective antagonistic compounds.

Acetylcholine in Physiological Regulation of Plants

Acetylcholine is a small neurotransmitter molecule reportedly involved in the regulation of growth and development by modulating the multitude of physio-biochemical processes in plants. Di Sansebastiano et al., (2014) [9] observed the synergistic effect of auxin and acetylcholine on the transcription of tomato expansin gene, *LeEXPA2* and on hypocotyl elongation, associated with the observed diversified effect on endomembranes. This elongation was prevented by adding to the medium the inhibitors of mammalian acetylcholine receptors. The sub-cellular target of acetylcholine signal is believed to be the vesicular transport. Involvement of acetylcholine in maintenance of water balance and photosynthesis has been demonstrated earlier [10]. The existence of choline acetyltransferase in blue-green algae suggests that acetylcholine was a biochemical necessity in the earliest photosynthetic organisms. Acetylcholine promotes seed germination, affects the growth of isolated plant organs and tissues, controls nyctinastic movement, membrane permeability to ions, phospholipid metabolism and respiratory processes, as well as regulates the activities of different enzymes like isoperoxidase, phenylalanine ammonia lyase and nitrate reductase. Acetylcholine also interacts with different phytohormones like auxins, ethylene and gibberellins, thereby affecting growth and development of plants [11]. Acetylcholinesterase plays a role in the gravity response of plants, and acetylcholine-mediated system has been proposed as a candidate for the potential-gating regulator. This system might achieve cell-to-cell transport of the hormone and other substances between the plant cells and might propagate a membrane depolarization [5]. Acetylcholine can mimic the action of red light in the regulation of some photomorphogenetic phenomena, so that acetylcholine serves as a secondary transmitter of phytochrome action or light-controlled signal transduction pathway. Light exposure promotes the synthesis of acetylcholine, which was undetected in darkness.

Acetylcholine in Plant Defense

Leaf spray and root application of acetylcholine during salt (150 mM NaCl) stress in *Nicotiana benthamiana* alleviated the inhibitions in gas-exchange parameters, chlorophyll content, antioxidative enzyme activities and leaf relative water content, by reducing the malondialdehyde content, enhancing the accumulation of osmolytes like proline and soluble sugars and activity of antioxidant enzymes. The most effective concentration of acetylcholine was 10 μM [12]. The same group [13] also reported the positive impact of application of 10 μM acetylcholine on the growth and chlorophyll metabolism in hydroponically grown *N. benthamiana* under salt (150 mM NaCl) stress. Acetylcholine improved the root hydraulic conductivity and relative water content, ameliorating the salinity-induced reduction in chlorophyll biosynthetic intermediates like protoporphyrin-IX, Mg-protoporphyrin-IX and protochlorophyllide, *via* up-regulation of *HEMA1, CHLH, CAO* and *POR* genes. Gas exchange parameters like stomatal conductance, internal CO_2 concentration and transpiration rate were also increased by acetylcholine thereby alleviating the salinity effects on photosynthesis. The salinity-induced enhancement in lipid peroxidation was declined due to acetylcholine treatment *via* modulations of the activities of antioxidant enzymes, reducing the uptake of Na^+ and increasing the uptake of K^+ resulting in declined Na^+/K^+ ratio. The role of acetylcholine in maintaining chlorophyll metabolism, photosynthesis, ion homeostasis, hydraulic conductivity and water balance was therefore established under salt stress. The *AchE* gene (encoding acetylcholinesterase) was identified in *Salicornia europaea* and the enzyme activity was increased in the root due to the accumulation of Na^+ and Cl^- from which acetylcholine-mediated depolarization of membranes and elimination of excess salt in the epidermal cells of roots was proposed by improving cell-to-cell transport of hormones and other metabolites [14]. Application of acetylcholine during osmotic stress imposed by mannitol treatment to the seeds of soybean (*Glycine max*), thereby subjecting them to two water potentials, -0.5 and -1.0 MPa, could improve the dry weight of shoot and root, total dry mass and seedling growth, even under severe osmotic stress [15]. The role of acetylcholine in plant root-shoot signal transduction was proposed by Hengbin et al., (2003) [16] based on their observation in *Vicia faba* seedlings under normal and water-stressed conditions. Under normal conditions, acetylcholine synthesized in roots is transported up to reach the guard cells for directly controlling the stomatal movement. Imposition of slight osmotic pressure decreased the content of acetylcholine in root tips and in the abaxial epidermis, correlated with the decrease in transpiration rate, thereby regulating stomatal closure. Therefore, plants transduce positive and negative signals among roots and shoots, co-ordinated by abscisic acid and acetylcholine to influence stomatal behavior, optimize plant growth under water deficiency, and adapt to variable environments. Application of 50 μM acetylcholine during cadmium (100 μM) stress in *N. benthamiana* revealed that acetylcholine alleviated Cd-induced reductions in plant growth, photosynthetic pigments and gas exchange attributes and improved the photosystem II activity, *via* maintaining the structural integrity of the chloroplasts and avoiding osmiophilic granule accumulation. Oxidative stress was minimized by lowering the cadmium accumulation *via* up regulating the enzymatic and non-enzymatic antioxidants, and phytochelatins [17]. The differences in acetylcholinesterase activity among cultivars from tropical and temperate zones are related to differences in ion conductivity and function of channel opening in cells. Heat stress was found to affect the enzyme activity in the primary and secondary pulvini of yard-long bean, petiole and root of radish, stem and node of cucumber [18]. In response to heat stress, acetylcholinesterase activity was found to be enhanced through post translational modification (glycosylation) in the coleoptile node and mesocotyl, with localization of activity in vascular bundles, endodermis and epidermis of coleoptile nodes and mesocotyls. Enhanced enzyme activity at extracellular space around epidermis is related to the regulation of water homeostasis during heat stress by preventing epidermal transpiration and controlling water and ionic balance [19]. In another observation, increased acetylcholinesterase activity were observed in nodes of heat-stressed maize seedlings, particularly in the whole endodermal cells between cortex and stele cells around vascular bundles, together with Ca^{2+} which can act as trigger for the release of the enzyme controlling ion channels [20]. Since acetylcholine with histamine causes the urticaria of nettle sting, the high acetylcholine content of nettles may provide protection against herbivores, thus conferring a selective mechanism for this characteristic [6].

Conclusion

Though it is still an underrepresented area of research as compared to its vertebrate and human counterparts, the field of acetylcholine research in plants is slowly emerging as a new area. The ability of this highly conserved molecule in promoting normal growth and physiology of plants as well as mitigate the effects of a diverse array of stresses highlights the application of this metabolite to battle against production challenges, rescuing plants from decreased yield and productivity, thereby ensuring food security. However, more exhaustive researches need to be conducted to determine the positive impact of acetylcholine during multiple environmental stresses as well as against infection by different pathogens. The involvement of acetylcholine in plant morphogenesis will also have potential implications for more studies on plant regeneration and developmental regulation.

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