

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(1): 1303-1309
www.biochemjournal.com
 Received: 16-11-2025
 Accepted: 19-12-2025

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Biochemical role of Phosphatidylcholine in acetylcholine synthesis and neuronal membrane integrity: A review study

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1p.7252>

Abstract

Background: Phosphatidylcholine (PC) is the most common phospholipid in the neuronal membrane and has a double role of maintaining the membrane structure and supplying choline to acetylcholine (ACh) production.

Aims: To summarize the biological synthesis between the PC metabolism and the ACh synthesis.

Methodology: Analytical review of the recent literature comprising of cellular and synaptosomal studies, animal models and clinical evidence was done to uncover the enzyme pathway and control in the release and use of choline in ACh synthesis.

Results: It has been demonstrated that phospholipase D (PLD) liberates choline out of PC to aid the production of ACh, especially in the case of exogenous choline deficiency, and PLD1 and PLD2 isoforms assume different roles in the regulation of this process. It has been demonstrated that membrane PC depletion causes membrane structural changes, such as membrane fluidity and protein activity alteration, which harm cell survival and growth. The balance of PC release of ACh holds the adequate levels without damaging the membrane, and that imbalance of the balance between choline release and renewal caused by lack of choline or high demand relates to the impaired neuronal functionality. In addition, therapeutic potential has been indicated by dietary interventions or cytokinin (CDP-choline) that do raise PC and ACh levels and preserve the neuronal membrane.

Conclusion: The PC balance between neuronal membrane and ACh synthesis is fundamental to the functioning of cholinergic neurons and derailment of this balance can be a cause of neurodegenerative conditions, which is why choline and phospholipid treatments of the neurons can help preserve neurons.

Keywords: Phosphatidylcholine, acetylcholine, choline, neuronal cells

1. Introduction

In the mammalian cell membranes, the most common phospholipid is Phosphatidylcholine (PC), or lecithin, which makes up approximately 40-50% phospholipids. PC plays an essential bifunctional role in neuronal tissue: it preserves the structural integrity of cellular membranes at the same time performing a metabolic role as the essential precursor of acetylcholine (ACh) synthesis, choline. It is where the duality creates a complex metabolic environment where the needs of the membrane maintenance and the synthesis of neurotransmitters need to be finely coordinated in cholinergic neurons [1, 2]. The first neurotransmitter characterized was ACh whose roles play central roles in cognition, consolidation of memory, attention, and motor control. Its synthesis is catalysed by choline acetyltransferase (ChAT), which is an enzyme whose activity cannot be substrate saturated in physiological conditions making the production of ACh dependent on the availability of choline [3]. Even though the extracellular choline can be endocytosed with high-affinity and low-affinity transporters, a new body of knowledge supports the idea that intracellular PC can be used as an alternative source of choline through enzyme hydrolysis [4, 5].

The functional resilience of cholinergic neurons has far-reaching consequences because the metabolic dependence of PC on ACh is interdependent. In cases where the extracellular choline is insufficient to support the ACh production, neurons can use the reserves of membrane PC, which might lead to the break of membrane integrity and cell performance [5, 6]. This metabolic susceptibility can be involved in the selective loss of cholinergic neurons that can be found in neurodegenerative disorders like the Alzheimer's disease [7].

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As a result, the present review summarizes the modern information on the biochemical processes that integrate PC metabolism with ACh synthesis and neuronal membrane stability, and examines the enzyme pathways, regulation processes, experimental results in a variety of model systems, and clinical implications of neurological diseases.

2. Biochemical Foundations of Phosphatidylcholine Metabolism

2.1 Biosynthesis via the CDP-Choline Pathway

In mammalian cells, PC is synthesized by the primary pathway: the CDP-choline pathway or Kennedy pathway. It is the rate limiting step in PC formation and it is a metabolic cascade of three consecutive enzymatic reactions [8-10]. Choline is first phosphorylated by the choline kinase to produce phosphocholine. This is followed by reaction of phosphocholine with cytidine triphosphate (CTP) under the action of the rate-limiting enzyme CTP:phosphocholine cytidyltransferase (CCT), to yield CDP-choline (cytidine diphosphate-choline or citicoline). Lastly, the choline phosphotransferase reversibly cleaves the phosphocholine group of CDP-choline to diacylglycerol (DAG), to produce PC [11]. The CDP-choline pathway is not merely a biosynthetic route but rather the pathway that is inseparably connected to cellular signalling and membrane homeostasis. It has been shown by Fagone and others that production of membrane phospholipids is regulated by metabolic flux through this cycle, and is associated with ACh production in neurons [3]. This cycle is reversible, whereby phosphomonoesters (phosphocholine and phosphoethanolamine) are the precursors and phosphodiester (glycerophosphocholine and glycerophosphoethanolamine) are the catabolites [11].

2.2 Alternative Synthetic Routes

PC may be assembled through different processes other than the CDP-choline pathway. A major pathway is the sequential methylation of phosphatidylethanolamine (PE) by phosphatidylethanolamine N-methyltransferase (PEMT) that adds three methyl groups to S-adenosylmethionine to PE, thus generating PC [5, 7, 12]. This pathway is particularly relevant in the hepatocytes and some of the neuronal subpopulations. The synthesis of ACh in human neuroblastoma cells may be based on choline produced endogenously by means of PC built by the PEMT pathway [5]. The third mechanism is the base-exchange pathway, which allows the head group of already existing phospholipids to undergo a direct replacement reaction to PC through the action of base-exchange enzymes [2, 13]. This pathway provides metabolic plasticity in the maintenance of PC under changing physiological conditions.

2.3 Choline Homeostasis

The choline homeostasis is a central regulatory crossroad between PC metabolism and the ACh synthesis. Choline has numerous crucial functions: as the precursor of ACh, as a component of membrane phospholipids (PC, sphingomyelin and plasmalogens), as a methyl donor after oxidation into betaine and as an emergent intracellular signalling molecule [14, 15]. The distribution of choline between these competing pathways need to be carefully regulated in order to maintain neurotransmitter production as well as membrane integrity [2, 12]. Both choline kinase (the first enzyme in PC synthesis) and ChAT (the enzyme catalysing ACh synthesis) do not

saturate on their substrates in physiological conditions, and thus make supplemental choline concomitantly stimulate both activities [6]. This results in a competitive relationship where the availability of choline will become the rate-limiting factor to the development of the membranes as well as the production of ACh. The active balance of choline flux between ACh and phospholipids is a reversible process that represents a primary regulatory process in cholinergic neurons [2].

3. Phosphatidylcholine as a Choline Reservoir for Acetylcholine Synthesis

3.1 Phospholipase D-Mediated Choline Liberation

The most significant finding in relation to the metabolic connection between phosphatidylcholine (PC) and acetylcholine (ACh) was the existence of the enzyme phospholipase D (PLD) as the one that releases choline on membrane PC to facilitate the production of acetylcholine. The initial studies of Hattori and other researchers incriminated synaptosomal PLD in the release of choline of PC, thus a direct metabolic linkage between membrane phospholipids and neurotransmitter biosynthesis was established [1]. Lee and co-authors were able to show conclusive findings that PLD-catalyzed PC hydrolysis is the source of choline precursor in ACh production in human neuronal cells. By pulsechase experiments involving radioisotopic labeling of 1-O-hexadecyl-2-hydroxy-sn-glycero(3)phospho[14C]choline, they were able to show that PC acted as a storage pool of choline that can be released via a single-step hydrolysis to free choline, bypassing any phosphocholine or glycerophosphocholine steps [9]. The released choline can then be changed to ACh and phosphocholine. Notably, experiments with hemicholinium-3, a blocker of choline transport proved that choline release happens intracellularly and does not rely on extracellular uptake of choline [9]. Blusztajn and colleagues also showed that, in the neuroblastoma of humans (LA-N-2) cells, ACh synthesis is possible when using choline, which was exchanged to PC, when the cells were flushed with choline-free media [5]. This observation defined that neurons have an inherent ability to mobilize membrane PC as a source of choline in cases when extracellular choline is not available.

3.2 Dynamic Equilibrium between Membrane Integrity and Neurotransmitter Synthesis

The use of membrane PC as a source of choline in ACh synthesis creates a metabolic burden between the maintenance of neurotransmitter synthesis and membrane integrity. Blusztajn *et al.* suggested that there was a dynamic balance of reversible fluxes of choline out of and into ACh and choline phospholipids which were controlled to ensure that ACh was produced in adequate amounts, and the functional integrity of membranes was not impaired [2]. Such a control system is necessary since the choline phospholipids are the largest lipid molecules of neuronal membranes.

Lack of the sufficient free choline to supply ACh may cause the mobilization of the PC reservoir to cause loss of PC in the membrane as well as other membrane constituents [6, 7]. Wurtman showed that in the case of the lack of free choline, the neurons take their own membranes and produce ACh. The effect of a long-lasting unbalance of availed choline versus the needed amount to produce ACh could be the disruption of the composition of the neuronal membranes or

the ability of the neuron to produce normal membranes, which could interfere with the functioning of neurons [7]. The experimental results provided by Blusztajn and others demonstrated that the changing of the PC content in NG 108-15 cells of the neuroblastoma x glioma hybrid cells influence cellular survival and growth rate which are unfavorable developments which suggests the negative impact on the viability since the neuronal membrane composition is changed [5]. This discovery indicates that PC levels are very vital in ensuring cellular health beyond being a source of choline.

3.3 Quantitative Contributions to the Acetylcholine Pool

The important role of PC-derived choline in the total amount of ACh has been determined in quantitative studies. Lee and colleagues established that the exogenous choline-independent channel of the PLD-contributed pathway in human neuronal cells LA-N-2 provides at least 15% of the total cellular ACh pool within one hour [9]. The significance of this large contribution is that this pathway is physiologically important, especially when the availability of choline is limited. Zhao and others have studied the particular isoforms of PLD involved in choline production in the cholinergic neurons. They discovered that PLD2 was the endogenous enzyme that mediated PC hydrolysis to generate ACh by using murine basal forebrain cholinergic SN56 cells. Antisense oligonucleotide downregulation of PLD2 decreased ACh levels by approximately 30%, mouse PLD2 overexpression raised basal PLD activity by 74% and ACh concentration by 26% [16]. Human PLD1 overexpression resulted in PLD activity upregulation 4.6-fold and ACh production upregulation 2.3-fold in the presence of phorbol 12-myristate 13-acetate (PMA), indicating that both forms of PLD could play a role in choline generation to ACh synthesis, although PLD2 seemed the major form under basal conditions [16].

4. Enzymatic Mechanisms and Molecular Pathways

4.1 Choline Acetyltransferase and Substrate Availability

Choline acetyltransferase (ChAT), the ACh synthesizing enzyme, is a catalyst which transfers acetyl group on acetyl-CoA to choline to produce ACh. One of the most important features of ChAT is the fact that it is not substrate-saturated under physiological conditions, ACh production, therefore, is directly linked to the presence of choline [6, 7]. This characteristic makes the cholinergic neurons especially vulnerable to changes in the choline supply and it is under this metabolic condition that the PC-derived choline gains physiological relevance. Wurtman and others showed that the intake of sufficient PC could elevate the levels of plasma choline many times, raising the levels of brain choline and saturating the substrate of ChAT [7]. This result has significant implications on the development of ways in which dietary choline and PC supplementation may affect the cholinergic neurotransmission.

The competitive distribution of choline between ACh production and PC biosynthesis also complicates the importance of the relationship between the ChAT activity and the availability of choline. The model of cholinergic neurons was implemented by Suidan and colleagues to show the symmetric distribution of choline between ACh and PC-generating pathway, and choline regulatory cues between PC-turnover and ACh generation [12]. The coordination of these processes is achieved by this metabolic coupling.

4.2 Phospholipase D Isoforms and Their Regulation

Phospholipase D has several isoforms, mostly, PLD1 and PLD2, which vary in terms of cellular localization, regulation, and physiological processes. PLD2 also seems to be the most abundant isoform in cholinergic neurons that produces choline at the expense of PC to form ACh in the absence of stimuli [16]. The PLD2 activity is constitutively active and localized on the plasma membrane, which puts it in the position to respond to the change in the demand of choline. PLD1 can also generate choline to be used in ACh synthesis, but is better controlled by protein kinase C (PKC) and needs phosphatidylinositol 4, 5-bisphosphate (PIP2) to maximize activity [16]. Zhao and others proved that the PLD1 overexpression significantly enhanced the ACh production in response to PMA, which is a PKC agonist, which indicates that PLD1 might play a larger role in the situations of increased neuronal activity [16].

PLD activity regulation is a key point of control between neuronal activity and the presence of choline to synthesize ACh. This process helps neurons to mobilize membrane PC as a reaction to a higher need of ACh production during the active phases of the synapses.

4.3 Metabolic Coupling in Cholinergic Neurons

The metabolic cascades that connect PC metabolism to the production of ACh are highly coupled, and have elaborate signals that align membrane maintenance and neuropathway synthesis. The CDP-choline pathway converting PC to PC, and the PLD-mediated hydrolysis pathway converting PC to choline coordinate with each other in order to ensure choline homeostasis [3, 14]. Citicoline (CDP-choline) is at the centre of this metabolic network. Citicoline synthesis as a step in PC construction is the rate-limiting step in PC synthesis [14]. Exogenous administration of citicoline may increase PC production as well as ACh production because it can be used as a choline donor in either of the two processes. The 90 days citicoline therapy of 90 days in old rats raised the levels of PC in the brain by 25% proving the ability of this pathway to react to the availability of substrates [14]. The process of enzyme activities is also metabolically coupled. CTP:phosphocholine cytidyltransferase (CCT), the rate-limiting enzyme in PC synthesis is activated by citicoline and controlled by the membrane lipid composition, hence forming a feedback loop in which the levels of PC in the membrane are compared with the PC biosynthetic capability [14]. This regulatory framework helps to have PC synthesis upregulated during membrane PC depletion keeping membrane integrity intact in spite of concomitant PC mobilization to ACh synthesis.

5. Phosphatidylcholine and Neuronal Membrane Integrity

5.1 Structural Role in Membrane Architecture

In addition to acting as a choline reservoir, phosphatidylcholine (PC) is also necessary in the structural roles of neuronal membranes. PC is the most abundant phospholipid in the mammalian cell membranes, which plays a role in mammalian cell membrane fluidity, curvature, and lipid microdomain organization [8, 17]. The biophysical characteristics of PC including its cylindrical molecular geometry and zwitterionic head group make it best adapted to the creation of stable bi-layer structures. PC is a component of a vital section of membrane signal transduction pathways, in which it is involved in the

production of second messengers which transmit extracellular signals into changes in cellular behavior [18]. Disturbances in the metabolism of phospholipids have the potential to disrupt these signalling cascades, and hence the importance of ensuring that neurons are not exposed to levels of PC that are lower than the norm. The integrity of PC in structures is particularly apparent in neurons, which have numerous membrane systems: axons, dendrites, and synaptic end terminals, which require a significant level of phospholipid synthesis and maintenance. Growdon highlighted that PC is a constituent part of the neuronal membranes and its depletion may impair the functioning of the membranes [8].

5.2 Consequences of Phosphatidylcholine Depletion

PC mobilization of acetylcholine (ACh) production can confer strong cytotoxics to membrane integrity and cellular functions. When the levels of choline are low, the use of membrane PC as a source of choline results in the loss of membrane phospholipids [5, 6]. This depletion will alter membrane composition, hence, altering fluidity, protein activity, and cell viability. Blusztajn *et al.* proved that PC content modification in NG 108-15 cells affects cellular survival and proliferation, and the shift in the neuronal membrane composition has an unwanted impact on the cell viability [5]. This fact shows that neurons have a limit to the degree of PC mobilization without interfering with the functioning of the cell. Wurtman hypothesized that sustained disproportion between choline supply and the amount of choline necessary to produce ACh might change the composition of the neuronal membrane or disrupt the ability of the neuron to generate normal membranes and this may negatively impact the neurons [7]. This metabolic vulnerability is an idea that can be used to aid in interpreting the selective degeneration of cholinergic neurons in some pathological states.

5.3 Membrane Phospholipid Turnover and Cellular Viability

The re-cycling of membrane phospholipids is a dynamic process which has to be highly controlled in order to maintain homeostasis of the cell. Ulus *et al.* showed that choline enhancement increases the release of ACh and works against the stimulation induced reduction of the phosphatidic contents under the rat corpus striatum membranes [19]. This is the protective effect which indicates that sufficient choline supply is necessary to maintain membrane phospholipid pools during periods of increased neuronal activity. Relationships between membrane PC turnover and ACh release were examined by Blusztajn and others in brain slices, synaptosomes and cultured cholinergic neurons [2]. With the help of their work, it was determined that the release of ACh is dependent on membrane PC turnover, and the higher the synthesis of ACh and its release, the higher the degradation of PC. This binding makes sure that the metabolic needs of neurotransmission have been satisfied, but also it predisposes the membrane to be depleted when long-term high activity is encountered. Conant and colleagues highlighted that citicoline has the ability to retain the structural and functional integrity of neuronal membranes especially in the case of low choline stores or high ACh demand, by preventing the breakdown of membrane phospholipids [14]. This protective effect makes

evident therapeutic possibilities of interventions that increase the supply of choline or PC production.

6. Experimental Evidence from Cellular and Animal Models

6.1 Synaptosomal Studies

Isolated nerve endings called synaptosomes which preserve most of the functional properties of intact synapses have been useful in characterizing the metabolic relationship between PC and ACh. Synaptosomal preparations were used to indicate phospholipase D (PLD) activity and its involvement in the provision of choline to make ACh [1]. These studies demonstrated that synaptosomal PLD may be stimulated by particular fatty acids and the monounsaturated fatty acids are more effective than the other species of fatty acids. Yavin and others examined the phospholipid derived choline intermediates and ACh production in mouse brain synaptosomes that established the metabolic mechanisms by which PC breakage is connected to ACh production [17]. Synaptosomal preparations have the advantage of maintaining sub cellular structure and enzymatic apparatus of nerve endings and also they enable to have control of experiment conditions explicitly.

6.2 Neuronal Cell Line Investigations

Neuronal cell lines have produced powerful models in their quest to probe the molecular processes that relate PC metabolism to the ACh synthesis. The human neuroblastoma LA-N-2 cells with cholinergic features have been widely utilized to show that ACh can be produced using choline obtained as a result of endogenous PC [5, 9]. These cells are able to be grown in media lacking choline allowing the researcher to isolate the role of intracellular PC-derived choline in ACh production. Blusztajn *et al.* demonstrated the use of LA-N-2, in which they perfused cells with choline free medium, and the cells still produce ACh using choline released by membrane PC [5]. This PC-dependent ACh synthesis was reduced by the use of tetrodotoxin, an inhibitor of sodium channels and neuronal activity, implying that the process is activity-dependent. The hybrid of neuroblastoma × glioma cells NG 108-15 cells were also useful in the investigation of the correlation between PC content and the cellular viability [5]. The cells bear both neuronal and glial characteristics and can be modified to change PC content thus supporting studies in the effects of PC depletion on cell survival and activity. Zhao and others adopted the use of murine basal forebrain cholinergic SN56 cells to determine PLD2 as the major isoform involved in the production of choline out of PC to produce ACh [16]. These cells were susceptible to molecular manipulations, such as the treatment with antisense oligonucleotides and transient transfection by expressing plasmids, allowing specific functions of PLD isoform to be studied and characterized in greater detail.

6.3 In vivo Animal Models

The use of animal models has played a necessary role in explaining the physiological significance of PC-ACh metabolic interactions in the brain as a whole. To clarify the point of the work, Ulus *et al.* employed the rat corpus striatum preparations to demonstrate that choline promotes the ACh release and aids in preventing the stimulation-enhanced decrease in the membrane phosphatidic levels [19]. These experiments found out that the metabolic coupling of

PC and ACh that is observed in isolated systems is also present *in vivo*. Conant and his colleagues examined the research in individuals in old age that proved that 90 days of citicoline treatment raised the levels of PC in the brain by 25%, thus illustrating the ability of CDP-choline route to the supplementation of substrates in the brain [14]. Gerbils and dogs have also been used to study the mechanism of citicoline on brain phospholipid metabolism and the cholinergic activity. Tests were done by Wurtman and others to determine the effect of dietary choline and PC on the levels of choline in the brain and the saturation of choline acetyltransferase substrate [7]. These works have shown that nutritional interventions could significantly alter the brain choline levels and by proxy the ACh production ability.

7. Clinical Implications and Therapeutic Applications

7.1 Alzheimer's Disease and Cholinergic Dysfunction

The Alzheimer's disease (AD) is characterized by progressive reduction in the cognitive ability along with the degeneration of cholinergic neurons, particularly in the basal forebrain. Cholinergic hypothesis of AD suggests that the impairment in this pathway is a significant contributor to the cognitive symptoms of AD. Therefore, the clarification of metabolic interdependence between phosphatidylcholine (PC) and acetylcholine (ACh) has both pathophysiological and therapeutic implications to a disease such as AD. Miatto *et al.* used the phosphorus-31 nuclear magnetic resonance (³¹P NMR) spectroscopy technique to identify aberrations in phospholipid metabolism in post-mortem AD brain tissue [11], and had found reduced phospholipase D (PLD) activity, which probably inhibit the release of choline out of PC and consequently inhibited ACh production. This decrease of PLD activity was associated with the variations in the phosphomonoester and phosphodiester levels, suggesting an increase in the phospholipid degradation or a compensatory mechanism of resynthesis [11]. Wurtman and others studied the role played by the concentrations of choline in regulating the synthesis of both ACh and PC, and they hypothesized that maladaptation of these two interconnected mechanisms might be the etiological basis of AD [6]. The increased susceptibility of cholinergic neurons in AD could be due to the fact that they have special metabolic requirements requiring not only high levels of ACh production but also vast amounts of membrane reorganization [13]. Recently, Conway *et al.* investigated the neuroprotective and neurotrophic effects of PC in AD, the relationship between the dietary intake of choline and cognitive performance, and suggested that PC could act not only as a provider of ACh but also due to its interaction with amyloid beta (Aβ) peptides in neuronal membranes [4].

7.2 Citicoline as a Therapeutic Agent

Citicoline (CDP-choline) has become an active therapeutic potential in a number of neurological issues due to its dual effects on the PC synthesis and ACh production. The clinical uses of citicoline in stroke and cognitive dysfunction in elderly were also reviewed by Conant *et al.* [14], who indicated its use as a replacement of choline, which helps maintain neuronal membrane integrity and neuronal functionality. The mechanism of action of citicoline has led to the development of a therapeutic premise; it supplies substrate to PC synthesis via the CDP-choline pathway in lieu of choline, thus maintaining membrane integrity; it

elevates choline to make ACh, supporting cholinergic neurotransmission; and it activates CTP:phosphocholine cytidyltransferase, the rate-limiting enzyme in the PC biosynthesis, thus promoting the whole process [14]. Identical evidence has been drawn to the efficacy of citicoline: in old rats, administration of citicoline increased brain PC levels by 25%, illustrating the ability of citicoline to increase brain phospholipid stores [14]. Human clinical studies have shown positively in cognitive, memory and post stroke neurological outcome improvements although not consistently, which necessitates more research studies to establish the best dosage regimens and patient selection criteria.

7.3 Dietary Choline and Cognitive Function

The identification of choline as a vital nutrient has prompted wide research on the dietary choline consumption and cognitive performance. Recently, Burns *et al.* have surveyed the role of choline as precursors of both ACh and phospholipids, and its health benefits [17]. There is a requirement of adequate dietary choline in keeping cholinergic neurotransmission and membrane integrity. Canty and others studied the role of lecithin and choline in human body and conclude that recent clinical reports support the claim that choline is crucial to normal liver and neurological functions [18]. The initiation of the right levels of intake by nutrition experts is indicative of increased recognition of its significance. Conway *et al.* examined the possible advantages of PC interventions on AD and cognition by measuring memory and learning effects [4]. Even though the effects of choline or PC supplementation as a cognitive enhancer are still inconclusive in a healthy population, there are benefits that could be obtained in cohorts of the population with a low-level of intake or increased metabolic rate. The dietary choline/brain choline/cognition relationship is multifactorial and is controlled by age, genetic polymorphisms of choline metabolism, and current nutritional status. Further studies are needed to determine those populations that are most likely to respond to supplementation and develop specific intervention guidelines.

8. Discussion

8.1 Integration of Metabolic and Structural Functions

The two functions of PC as a structural component of neuronal membranes and a metabolic store of choline is a beautiful but potentially problematic metabolic solution. This dualistic quality provides a basic conflict between homeostasis of neurotransmitter production and maintenance of membrane integrity. It has been shown that neurons have developed elaborate regulatory processes to deal with this tension, and they include various synthetic pathways of PC, dynamic balance between choline release to ACh and PC, as well as highly regulated PLD activity [2, 12]. The metabolic coupling between PC synthesis and degradation makes sure that the levels of membrane PC are kept within physiologically viable levels. CDP-choline pathway is the major pathway to PC synthesis and CCT is the rate-limiting enzyme that is regulated by the composition of membrane lipids [3, 14]. The depletion of membrane PC increases CCT activity which enhances PC production. On the other hand, neuronal activity and choline requirement regulate PLD-mediated hydrolysis of PC to ensure that membrane PC is mobilized only when it is needed in the production of ACh [16]. This type of regulatory

structure tends to maintain homeostasis in normal physiological conditions. Nevertheless, in the presence of a deficiency of choline in the environment, overload of the metabolic processes, or disease conditions, this balance may be disrupted, which leads to the depletion of membrane PC and possible dysfunction of cells ^[5-7].

8.2 Selective Vulnerability of Cholinergic Neurons

Wurtman hypothesized that the choline metabolism is the basis of selective vulnerability to cholinergic neurons ^[13]. These neurons are faced with unique metabolic pressures due to their heavy workload in the production of ACh and maintenance of the membrane. The axonal and dendritic arborescences of much of the cholinergic neurons are large and form a great area of membrane that must be maintained all the time. At the same time, they have to maintain large amounts of ACh production to facilitate neurotransmission. This two-fold metabolic load makes cholinergic neurons highly vulnerable to the conditions that reduce the amount of choline or interfere with the PC metabolism. Combined with impaired PLD activity ^[11], disturbed phospholipid metabolism, and increased metabolic stress, all these factors may create the conditions in which cholinergic neurons could no longer support adequate production of ACh and membrane integrity, which may explain their selective degeneration in AD. The metabolic vulnerability concept provides a paradigm of why cholinergic neurons are selectively insulted in particular neurodegenerative diseases and proposes that any intervention that influences the supply of choline or PC metabolism can confer neuroprotection. This is the basis on which citicoline and other compounds containing choline can be used therapeutically ^[14].

8.3 Methodological Considerations and Limitations

The reviewed studies have used a wide range of experimental designs, which have their own limitations and advantages. Synaptosomal preparations maintain the subcellular structure of nerve endings but do not include the cellular environment of intact cells ^[1]. Neuronal cell lines are potent model systems of molecular studies but with limited ability to reproduce primary neuron properties ^[5, 9, 16]. Animal models are more physiologically relevant but are more difficult to experiment with ^[19]. Radioisotopic labeling methods have been very useful in tracking metabolic routes and in determining the role of PC-derived choline in ACh synthesis ^[9], but the experiments are typically short-term and might be inadequate in showing long-term metabolic changes. NMR spectroscopy provides a non-invasive evaluation of metabolism of phospholipids but has a low sensitivity and spatial resolution ^[11]. Most studies focus on acute choline depletion or PC mobilization, but there are few studies on the consequences of chronic PC-ACh metabolic abnormalities. To apply experimental results on cell culture or animal-based models to human neurological diseases, special consideration should be taken in interpreting the results due to species-specific variations in the choline metabolism and PC synthesis pathways. Further studies using state-of-the-art lipidomics, metabolomics, and in-vivo imaging can result in a more detailed representation of PC-ACh metabolic interrelationships in intact brain tissue in physiological and pathological conditions.

9. Future Directions

There are also a number of critical questions that have not been answered about the role of PC in the biochemical

mechanism of neuron production of ACh and their maintenance of the neuronal membrane integrity. To begin with, specific regulatory processes that control PC synthesis, degradation, and choline distribution between the ACh and PC pathways need to be better defined. Although PLD2 is the most frequently observed isoform involved in the generation of choline used in the production of ACh ^[20], the signal transduction processes regulating the action of PLD2 in neuronal activity and choline supply are poorly defined. Second, the chronic PC-ACh metabolic imbalances have some long-term effects that should be investigated. Majority of the experimental studies have concentrated on the acute effects, when neurodegenerative diseases develop over years or decades. In animal models considering the effects of chronic inadequacy of choline or perturbed PC metabolism on neuronal morphology, activity and survival would be invaluable. Third, the targeting PC-ACh metabolism has a therapeutic potential that still needs additional clinical assessment. Citicoline has been promising in certain studies; still, there are several issues in the formulation of optimal dosages, selection criteria of patients to respond to, and the combination of citicoline and other therapeutic modalities. There are novel approaches to use like selective PLD modulators or directing choline containing compounds to cholinergic neurons which would be better than the existing ones. Fourth, there is an opportunity to investigate the role of PC and choline in metabolism in other neurological and psychiatric disorders other than AD. Cholinergic dysfunction has been suggested in the pathogenesis of schizophrenia and depression, and a better understanding of the metabolic pathway of this dysfunction may trigger novel treatments. Fifth, the individual difference in the choline requirement and metabolism of PC that may be associated with genetic polymorphisms in enzymes of these pathways, require exploration. Individualized supplementation schemes on the foundation of individual metabolism could be more effective than mass measures. Lastly, the connection between PC metabolism and other aspects of neuronal lipid metabolism, such as sphingolipids, plasmalogens, and lipid signaling molecules, is another vital field of future studies. The complex lipid structures in neurons and PC metabolism cannot exist in isolation, but rather, it interacts with the rest of the lipid homeostatic processes ^[21].

10. Conclusion

Phosphatidylcholine (PC) is an important constituent of neuronal metabolism, functioning as an important structural constituent of membranes and as a metabolic store of choline to be used in the production of acetylcholine (ACh). PC hydrolysis by phospholipase D (PLD) is a source of important choline especially where intracellular choline is low. This metabolic interdependence ensures that there is a dynamic balance of production of the neurotransmitters and the integrity of the membrane. This balance is regulated by enzymatic pathways, such as the CDP-choline cycle, hydrolysis mediated by PLD, and choline acetyltransferase activity, in order to favour cholinergic neurotransmission in the normal condition. Nonetheless, low choline diet, metabolic stress or disease may destroy this homeostasis, draining membrane PC and compromising neuronal activity. This vulnerability may be the cause of selective degeneration of cholinergic neurons that are found in Alzheimer disease and other neurodegenerative disorders. Cytokoline and other choline derivatives have proven to be

effective therapeutic agents to boost PC synthesis and ACh production to provide neuroprotection. Additional studies are required to streamline these methods and work on specific treatments that would deal with PC-ACh metabolism.

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