CURRENT ASPECTS REGARDING THE ACETYLCHOLINE METABOLISM

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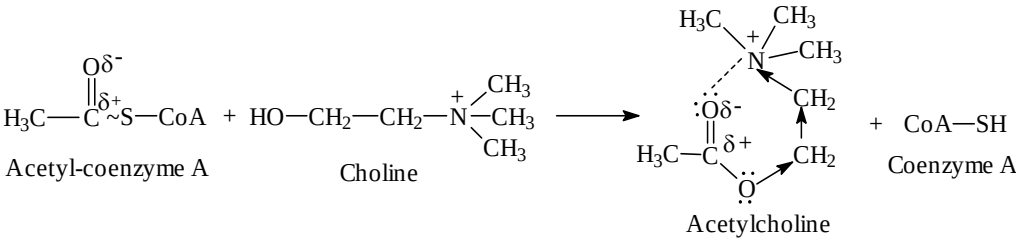
Abstract: In the last 30 years, many biochemistry and neuroscience studies elucidated the main aspects of the acetylcholine metabolism, including proteins responsible for the biosynthesis, storage, release and degradation of acetylcholine. In this review, some data about the precursors implicated in acetylcholine biosynthesis and about some characteristics of cholinergic system enzymes, choline acetyltransferase and acetylcholinesterase, will be discussed.

INTRODUCTION

Acetylcholine (ACh) is well known as a neurotransmitter in both the central and peripheral nervous systems of mammalian species [Ciobîcă, 2008], modulating the activity of the cholinergic synapses. It is found in almost every structure of the nervous system, but also in some non-neuronal structures. According to Webster [Webster, 2005] acetylcholine is the most important neurotransmitter outside the CNS (central nervous system), whether this is at sympathetic or parasympathetic ganglia of the autonomic nervous system, the adrenal medulla or the neuromuscular junctions in the skeletal muscle.

BIOSYNTHESIS OF ACETYLCHOLINE

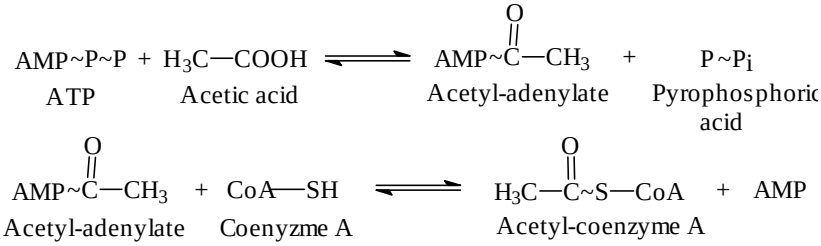
Acetylcholine (ACh) is an ester of the acetic acid with the quaternary ammonium alcohol choline. It is biosynthesized in the cholinergic neurons and other cell types by the choline O-acetyltransferase (ChAT, also called choline acetylase by Nachmansohn and Machado in 1943 [Artenie, 1966] ; EC 2.3.1.6), the enzyme-catalyzed transfer of acetyl group from acetyl-coenzyme A to choline, according to the following reaction :



Various aspects regarding the biosynthesis of ACh are presented in many research articles, reviews [Hamitov, 1967 ; Artenie, 1968 ; Hebb, 1972 ; Chao, 1980] and books [Tuček, 1978].

Acetyl-coenzyme A can be formed as a result of many reactions in the cell, including acetate activation, oxidative decarboxilation of pyruvate, citrate cleavage etc. [Korkes et al., 1952 ; Tuček et al., 1970].

Acetate activation is a complex process, catalyzed by acetyl-coenzyme A synthetase [acetate : CoA – ligase(AMP), EC 6.2.1.1]. The mechanism involved in the activation of acetate was first discovered by Paul Berg [Berg, 1955 ; Berg, 1956]. He demonstrated that the formation of acetyl-CoA from acetic acid, ATP and coenzyme A is made according to the following mechanism, having acetyl-adenylate as an intermediary product:



The oxidative decarboxilation of pyruvate generates acetyl-CoA in a multiple-step process (figure 1) that is nexus of central metabolism and is realized under the action of **the pyruvate dehydrogenase multienzyme assembly** [Artenie, 1976 ; Garrett and Grisham, 1995].

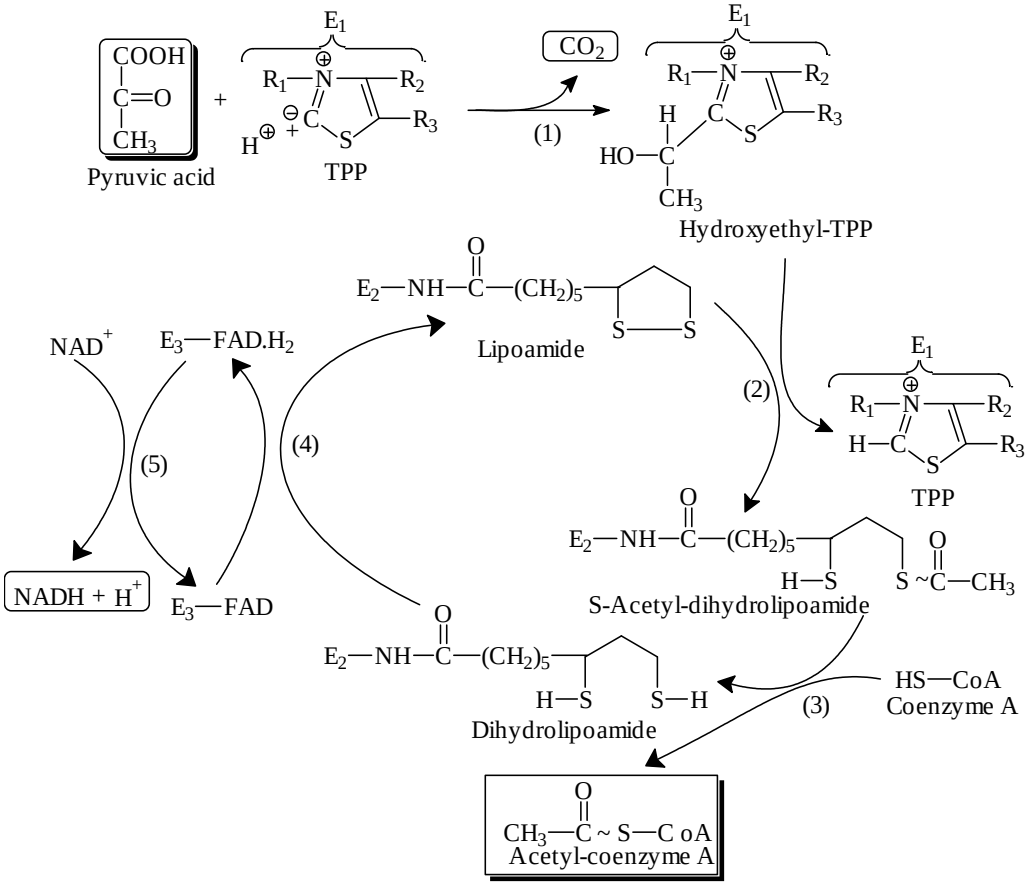


Figure 1. The mechanism of the pyruvate dehydrogenase reaction(Adapted from Artenie, 1976 and Garrett and Grisham,1995).

E_1 – Pyruvate dehydrogenase(EC 1.2.4.1) ;

E_2 – Dihydrolipoyl transacetylase(EC 2.3.1.12) ;

E_3 – Dihydrolipoyl dehydrogenase(EC 1.8.1.4).

As a result of the (1) – (5) reactions from the Figure 1, the oxidative decarboxilation of the pyruvate take place, processes which is coupled with the attaching of acetyl on coenzyme A and the reduction of NAD^+ to NADH:

$$CH_3COCOOH + HS\text{-}CoA + NAD^+ \rightarrow CH_3\text{-}CO\sim S\text{-}CoA + NADH + H^+ + CO_2$$

Pyruvic acid Acetyl – CoA

Acetyl-CoA is synthesized by pyruvate dehydrogenase complex(PDHC) in the mitochondria of all cells. Most of the acetyl-CoA produced by this process is used to generate ATP in the tricarboxylic acid cycle. However, in cholinergic cells, some of this acetyl-CoA is transported across the mitochondrial membrane into the cytoplasm, in order to be used for the biosynthesis of ACh.

The activity of PDHC, which provides acetyl-CoA for ACh biosynthesis in the white matter from the pig brain, ranged between 33-50% of the hippocampal activity. At the same time, the activity in the caudate nucleus was similar to activity in the hippocampus and other gray structures [Hassel et al., 2008].

The reaction cleavage of citrate is catalyzed by the citrate cleavage enzyme [EC 4.1.3.8; ATP:citrate oxaloacetate-lyase(coenzyme A-acetylating and ATP-dephosphorylating)], also known as ATP-citrate lyase, the enzyme that converts glucose-derived citrate into acetyl-CoA and oxalocetate (Eq. 1), as shown by Srere and collaborators [Srere et al., 1953 ; Srere, 1963 ; Srere et al.,1964] :

$$ATP + citrate + CoA \underset{}{\overset{Mg^{++}}{\rightleftharpoons}} acetyl\text{-}CoA + oxaloacetate + ADP + P_i \quad \text{(Eq. 1)}$$

Regarding the physiological functions of the citrate cleavage, it is believed that in the nervous tissue this way of forming acetyl-CoA could result in acetyl which is used for the acetylation of choline [Wollemann, 1956].

Choline (trimethyl-beta-hydroxyethylammonium) is found in animal and human tissues and can have two different origins: endogenous, formed by biosynthesis and exogenus, resulted from the food intake.

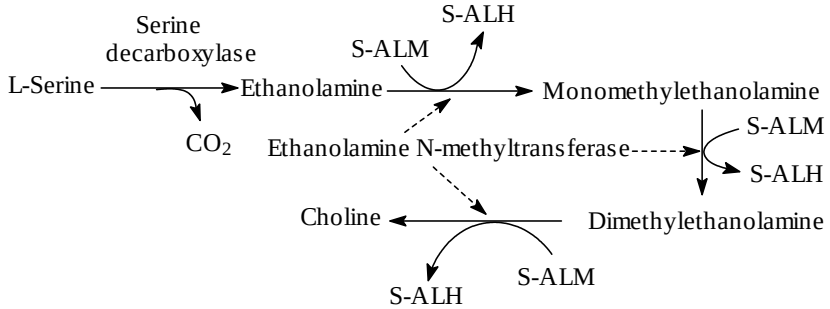


Figure 2. Pathway of choline biosynthesis from L-serine
S-ALM : S-Adenosyl-L-methionine
S-ALH : S-Adenosyl-L-homocysteine

Choline is biosynthesized in animal tissues from the amino acid L-serine, through several different steps (figure 2). The carbon chain of serine is used in the synthesis of the ethanolamine moiety of choline. The first step in choline biosynthesis is direct decarboxylation of L-serine to ethanolamine, which is catalyzed by a serine decarboxilase. Consecutively, choline is

formed by three N-methylation steps of the ethanolamine. All three methyls from the choline are formed from the S-adenosyl-L-methionine [Bremer et al., 1960].

Greenberg and his associates showed that phosphatidylserine is decarboxilated, giving rise to phosphatidyl-ethanolamine (figure 3), which is methylated stepwise to phosphatidyl-choline, which could result in choline formation [Bremer et al., 1960].

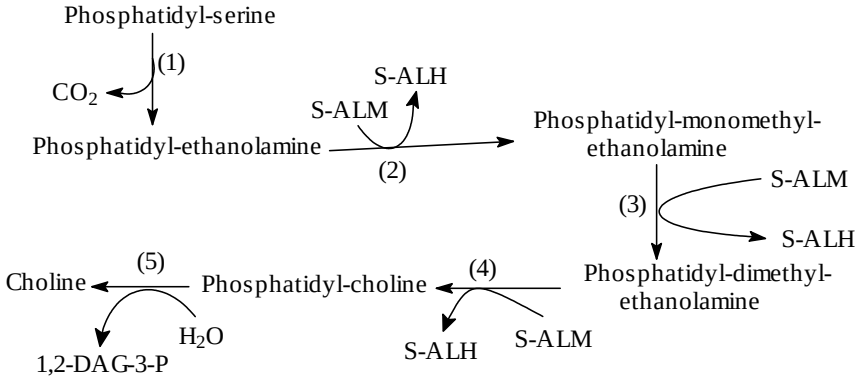


Figure 3. Pathway of choline biosynthesis from phosphatidyl-serine
S-ALM : S-Adenosyl-L-methionine
S-ALH : S-Adenosyl-L-homocysteine
(1) : Phosphatidyl-serine decarboxylase
(2), (3) and (4) : Phosphatidyl-ethanolamine methyltransferase
(5) : Phospholipase D

Results obtained after 1985 demonstrated that cholinergic neurons utilize choline stored in phosphatidylcholine to biosynthesize ACh [Blusztajn et al., 1987].

Most of the choline used for the formation of ACh is recycled following the enzymatic hydrolysis of ACh in the synaptic space. Also, relatively recent, Löffelholz [Löffelholz, 1998] reported that in the brain, choline is ultimately provided by the circulation.

ACh is synthesized at the citoplasmic level of the neuronal body (20 %), but most of the synthesis process is made in the terminal axon (80%) and then is predominantly stored in specific cytoplasmic structures called synaptic vesicles.

The majority of ACh in rat cultured sympathetic neurons is biosynthesized in distal axons/axon terminals from choline taken up by a high-affinity choline transporter in distal axons [Bussière et al., 2001].

Choline acetyltransferase (ChAT) is also biosynthesized in the cholinergic neuron. ChAT is distributed in the cardiac auricles of rats, rabbits, cats and guinea-pigs [Tuček, 1964], also in the sarcoplasma and the proteins of myocardium, but also in the rat skeletal muscles [Esîrev et al., 1968].

ChAT of rabbit brain has been partially purified and characterized [Severin and Artenie, 1967]. Purified preparations of ChAT contain 3-5 SH-groups per enzyme molecule. Usually, thiol enzymes are labile and less stable in solution or in purified state. Addition of cysteine and/or imidazole to the choline acetyltransferase from the brain of rabbits during storage of enzyme purified protects the enzyme from the loss of activity [Severin, Artenie, 1968]. Also, evidence has been accumulated showing the essential involvement of an imidazole group in the active site of ChAT [Burt et al., 1973 ; White et al., 1970].

Results obtained with gel filtration on Sephadex G-200, G-100 Superfine indicated that the sodium phosphate- and Triton DN-65-solubilized fractions of ChAT, partially purified from a nerve ending fraction of rat forebrain, had molecular weights that range from 73,000 to 78,000 Da, whereas the NaCl-solubilized fraction of ChAT had a molecular weight that range from 230,000 to 240,000 Da [Badamchian, Carroll, 1985]. Using SDS-PAGE and Western blotting the same authors [Badamchian, Carroll, 1985] reported that all three fractions of ChAT were composed of the same non-identical subunits.

The two isozymes of ChAT from head ganglia of *Loligo pealei* have been examined by PAGE, gel chromatography and equilibrium sedimentation in the ultracentrifuge [Polsky, Shuster, 1976]. Each isozymes appeared to contain two non-identical catalytically active subunits, with molecular weights of approx. 37,000 and 56,000 Da.

However, the existence of several isoforms of ChAT in the brain of some vertebrate species has not been confirmed by research conducted by Chao et al. [Chao, 1980].

ChAT activity from the white matter of pig brain represented 10-15% of the hippocampal value, the highest activity being found in fimbria [Hassel et al., 2008].

Moreover, using Northern blot or RT-PCR (reverse transcription-polymerase chain reaction), expression of ChAT has been identified in human blood mononuclear leukocytes, human leukemic T-cell lines and rat lymphocytes [Kawashima, Fujii,2000].

In addition, the study of Massarelli et al.[Massarelli et al.,1977] reports a diurnal variation of ChAT activity in human blood : the maximum enzyme activity appeared to be localized in the afternoon hours and the minimum in the morning hours. Also, ACh, ChAT, AChE and muscarinic receptors in the mouse brain showed a clear daily rhythm [Pan S.Y., 1991].

REGULATION OF ACETYLCHOLINE (ACh) BIOSYNTHESIS

ACh biosynthesis is regulated by the availability of precursors acetyl-coenzyme A and choline, or by the activity of choline acetyltransferase (ChAT).

Availability of acetyl-coenzyme A. Some interesting investigations regarding the source of acetyl-CoA used for the biosynthesis of ACh in brain were reviewed by Jope [Jope, 1979]. Three sources of acetyl-CoA have received support : citrate conversion catalyzed by citrate lyase, direct release of acetyl-CoA from mitochondria following its synthesis from pyruvate by pyruvate dehidrogenase complex(PDHC), and production of acetyl-CoA by cytoplasmic PDHC. In addition, investigations indicating that acetyl-CoA availability may limit ACh synthesis are currently reviewed.

Availability of choline. Choline for ACh biosynthesis can potentially be supplied through three patways: (1) *de novo* synthesis, (2) uptake of free choline by low- and high-affinity transporters and (3) turnover of membrane phosphatidylcholine.

Mullen and collaborators [Mullen et al., 2007] stated that *de novo* synthesis of choline is important for ACh production in any organism. Additionally, choline is naturally produced by the liver.

Free choline circulating in blood plasma readily crosses the blood-brain barrier and is taken up by the cholinergic nerve terminals, mostly by high-affinity plasma-membrane choline transporters (CHT), which are temperature-, energy- and sodium-dependent. These transporters are the primary means by which choline needed for the biosynthesis of ACh is carried into the neuron. The active uptake of choline is considered to be the rate-determinating step in production

of the ACh [Haga and Noda, 1973 ; Guyenet et al., 1973 ; Barker and Mittag, 1975 ; Mulder et al., 1974]. The existence of a high-affinity CHT activity in rat brain synaptosomes was first reported more 45 years ago [Yamamura and Snyder, 1972 ; Haga and Noda, 1973].

Recently, the molecular entity for the high-affinity choline transporter in human was identified and is designated as CHT1 [Okuda, Haga, 2000; Okuda, Haga, 2003]. CHT1 mediates Na^+- and Cl^--dependent choline uptake with high sensitivity to hemicholinium-3. CHT1 has been characterized both at the molecular and functional levels and was confirmed to be specifically expressed in cholinergic neurons [Okuda, Haga, 2003]. Similarly, CHT proteins in rat, mice and other animals are expressed in most cholinergic neurons [Misawa et al., 2001; Ferguson et al., 2003].

In vertebrates there are also low-affinity choline transporters which take part to choline transportation into the presynaptic nerve terminal. But the high-affinity choline transporters, inhibited by hemicholinium, are probably responsible for most of the choline recycled from the synapse and used to form ACh.

ACh concentrations in the whole rat brain or in different brain regions and free choline concentrations in the blood serum and brain vary with dietary choline consumption [Cohen, Wurtman, 1976]. Choline is a compound that can be obtained from foods such as egg yolks, kidney, liver, seeds, legumes and various vegetables.

Functional regulation of ChAT. ChAT is biosynthesized in the cholinergic neurons and regulates ACh biosynthesis under some specific conditions. These include competitive inhibition of ChAT by ACh feedback [Kaita and Goldberg, 1969 ; Morris et al., 1971] and mass action regulation of ChAT [Glover and Potter, 1971].

Posttranslational phosphorylation is a common mechanism for regulating the function of proteins. Analysis of the primary structure of 69-kDa human ChAT indicates that it has putative phosphorylation consensus sequences for multiple protein kinases. ChAT is phosphorylated on serine-440 and threonine-465 by protein kinase C and calmodulin kinase II, respectively. These phosphorylation events regulate activity of the enzyme, as well as its binding to plasma membrane and interaction with other cellular proteins [Dobransky, Rylett, 2003].

STORAGE OF ACh IN VESICLES

After its biosynthesis, most ACh is packaged into cytosolic storage vesicles located in the presynaptic nerve endings. ACh is transported from the cytoplasm into individual vesicles through a carrier protein on the vesicle membrane called the vesicular ACh transporter. This couples an influx of ACh with an efflux of proton. ACh is stored at the nerve ending until an action potential arrives and allows for its release into the synaptic cleft. Only the stored form of ACh serves as the functional neurotransmitter.

HYDROLYSIS OF ACh

The inactivation of ACh is a necessary process, which takes place in the synaptic and presynaptic regions, on a very short period of time. Most of ACh is degraded in the synaptic fluid under the action of a specific enzyme, called **acetylcholinesterase** (AChE, EC 3.1.1.7). AChE, which is present in the nervous tissue and erythrocytes of animal organism, degrades ACh, through its hydrolytic activity, producing choline and acetate, as shown below (figure 4).

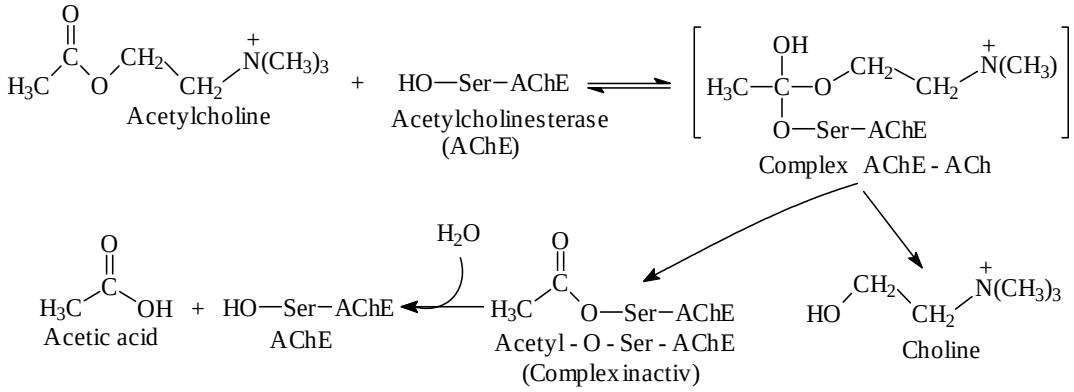


Figure 4. Mechanistic steps in the hydrolysis of ACh [Adapted from Soreq et al., 2001].

As seen in figure 4, AChE promotes ACh hydrolysis by forming a complex intermediate enzyme-substrate, AChE-Ser-O-ACh, with the release of choline and an inactive complex called acetyl-O-Ser-AChE. Subsequently, by the hydrolysis of this complex, AChE and acetic acid are released. The active serine belongs to the catalytic triad Glu-His-Ser. Also, the catalytic turnover rate of AChE is very high.

A molecule of AChE can inactivate about 10,000 of ACh molecules per second. Almost half of the ACh molecules that are released in the synaptic cleft together with the nervous impulse are inactivated by hydrolysis into choline and acetate, before reaching the receptors. The speed of ACh inactivation is about 10.7 seconds. However, this time is sufficient for the rest of ACh molecules to be coupled to post-synaptic cholinergic receptors and trigger processes characteristic of each of these receptors [Greenfield, 1991].

The degree of ACh inactivation under the action of AChE, determines the functional state of cholinergic nerve function and the cholinergic expression.

Thus AChE is a key enzyme for cholinergic transmission, which plays a vital role in the nervous system and in the neuromuscular junction of vertebrates, where its catalytic activity serves to terminate synaptic transmission.

In humans, AChE is encoded by the single AChE gene [Ehrlich et al., 1992].

AChE is the target of many natural and synthetic compounds, known as acetylcholinesterase inhibitors. Inhibition of AChE increases the concentration of ACh in the synapse and results in production of both muscarinic and nicotinic responses. Most of the acetylcholinesterase inhibitors include cholinergic drugs, nerve gases and particularly organophosphates and insecticides. In addition acetylcholinesterase inhibitors (AChEI) are also referring as anticholinesterases. The classic AChEI is eserine (physostigmine), an alkaloid obtained from the seeds of the Calabar bean, *Physostigma venenosum* [Golikov et al., 1964]. The uses of these drugs have been also suggested for the treatment of Alzheimer’s disease and similar cognitive disorders [John et al., 1993], which are characterized by a massive cholinergic deficiency at the cortex or basal forebrain level.

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