Chemico-Biological Interactions 203 (2013) 113–119

Contents lists available at SciVerse ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Cholinergic involvement and manipulation approaches in multiple system disorders

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ARTICLE INFO

Article history:
Available online 14 August 2012

Keywords:
Cholinesterase
microRNA
Organophosphate poisoning

ABSTRACT

Within the autonomic system, acetylcholine signaling contributes simultaneously and interactively to cognitive, behavioral, muscle and immune functions. Therefore, manipulating cholinergic parameters such as the activities of the acetylcholine hydrolyzing enzymes in body fluids or the corresponding transcript levels in blood leukocytes can change the global status of the autonomic system in treated individuals. Specifically, cholinesterase activities are subject to rapid and effective changes. The enzyme activity baseline increases with age and body mass index and depends on gender and ethnic origin. Also, the corresponding DNA (for detecting mutations) and RNA (for measuring specific mRNA transcripts) of cholinergic genes present individual variability. In leukocytes, acetylcholine inhibits the production of pro-inflammatory cytokines, suggesting relevance of cholinergic parameters to both the basal levels and to disease-induced inflammation. Inversely, acetylcholine levels increase under various stress stimuli, inducing changes in autonomic system molecules (e.g., pro-inflammatory cytokines) which can penetrate the brain; therefore, manipulating these levels can also effect brain reactions, mainly of anxiety, depression and pain. Additionally, neurodegenerative diseases often involve exacerbated inflammation, depression and anxiety, providing a focus interest group for cholinergic manipulations. In Alzheimer's disease, the systemic cholinergic impairments reflect premature death of cholinergic neurons. The decline of cholinesterases in the serum of Parkinson's disease and post- stroke patients, discovery of the relevant microRNAs and the growing range of use of anticholinesterase medications all call for critical re-inspection of established and novel approaches for manipulating cholinergic parameters.

1. Introduction

Nerves transmit their impulses across most synapses and neuroeffector junctions by means of specific chemical agents known as neurohumoral transmitters or, simply, neurotransmitters. The cholinergic system is best characterized as a neurotransmitter-derived signaling pathway which functions within the nervous system and between neurons and their effectors. Acetylcholine (ACh) is the principal neurotransmitter of brain cholinergic neurons and postganglionic parasympathetic and cholinergic sympathetic nerves, in the periphery, and of both sympathetic and parasympathetic preganglionic fibers [1]. ACh determines channel opening at neuromuscular junctions (NMJs) of skeletal muscles [2]. Yet more recently, various peripheral cells such as pancreatic alpha cells [3], endothelial [4] and placenta cells were found to express non-neuronal ACh [5]. In the circulation, cholinergic molecules have been detected in thrombocytes [6], and lymphocytes [7]. Correspondingly, cholinergic signaling is simultaneously involved in central cognitive processes such as learning [8] and memory [9], while striving for peripheral homeostasis through activation of the parasympathetic system and mediating both neuromuscular [10] and inflammatory responses [11]. Over the last century, much effort has been devoted by many to develop reliable methods for manipulating cholinergic signaling and measuring its values as biomarkers distinguishing between health and disease; and the recently prolonged life expectancy and the expanded use of anticholinesterase medications (e.g., in Alzheimer's disease) call for re-inspecting the knowhow on manipulating of cholinergic parameters. In the following, we provide a partial cover of this massive effort (due to space limitations), with focus on recent

Abbreviations: AcCoA, acetyl-coenzyme A; ACh, acetylcholine; AChE, acetylcholinesterase; AChR, ACh receptors; AD, Alzheimer's disease; ANS, autonomic nervous system; AThCh, acetylthiocholine; BAPTA-AM, 1,2-bis-(2-aminophenoxy)ethane *N,N,N,N′*-tetraaceticacid tetra(acetoxymethyl)ester; BChE, butyrylcholinesterase; BThCh, butyrylthiocholine; BW284C61, di(p-allyl-N-dimethylaminophenyl)pentane-9-one bromide; Ch, choline; ChAT, choline acetyltransferase; CHT, choline uptake transporters; CNS, central nervous system; CSF, cerebrospinal fluid; DTNB, dithionitro-benzoate; IL, interleukin; iso-OMPA, tetra-isopropyl-pyrophosphoramide; mAChR, muscarinic ACh receptors; MG, myasthenia gravis; miR, microRNA; nAChR, nicotinic ACh receptors; NMJ, neuromuscular junctions; OP, organophosphate; PVN, paraventricular hypothalamic nucleus; SSRI's, selective serotonine reuptake inhibitors; TCAs, tricyclic antidepressants; TNB, 3-carboxy-4-nitrobenzenthiolate anion; VAChT, vesicular ACh transporter.

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http://dx.doi.org/10.1016/j.cbi.2012.07.007

developments and a limited selection of the relevant bibliography [See Supplementary material for more bibliography for this and the following sections].

1.1. Acetylcholine signaling and its cognitive, behavioral, muscle and immune functions, the corresponding genes and proteins, non-neuronal cholinergic signaling

ACh-derived choline and acetyl-coenzyme A (AcCoA) together, give rise to ACh in the presynaptic terminal, catalyzed by choline acetyltransferase (ChAT, Graphical abstract). The vesicular ACh transporter (VAChT), which is encoded from the first intron in the ChAT gene and is hence co-regulated with it packs newly synthesized ACh into vesicles, and the corresponding cholinergic terminals stably contain micromolar concentrations of basal choline. In body fluids, peripheral cells and organs and neuronal elements, the action of ACh is terminated by its degradation into choline and acetate by two closely homologous enzymes, acetyl- and butyrylcholinesterase (AChE, BChE) [12]. AChE gene expression undergoes several regulation levels; alternative splicing allows the production of three C-terminally distinct AChE variants,- the 'synaptic' (S), 'erythrocytic' (E) and 'readthrough' (R) AChE isoforms. At the post-transcriptional level, AChE undergoes silencing by microRNA (miR)-132 [13], and perhaps other microRNAs with both neuronal and immune functions [14,15].

When AChE hydrolyses ACh, ACh-derived choline is rapidly cleared from the extracellular space and recycled into presynaptic terminals via hemicholinium-3-sensitive, high-affinity choline uptake transporters (CHT). Once released, ACh acts on either muscarinic or nicotinic ACh receptors (AChR), depending on the target innervated. Brain nicotinic receptors can exist as heteromeric combinations of α (2–10) and β(2–4) subunits, and as α 7 homopentamers. In muscle-type receptors, the non- α subunits are β1, γ or ε, and δ. Nicotinic receptors are pentamers, and each nAChR subtype exhibits distinct biophysical and pharmacological properties. Muscarinic receptors consist of five subtypes, M_1-M_5 and belong to the class of heptahelical G protein-coupled metabotropic receptors. M1, M3 and M5 couple preferentially to the Pertussis toxin-insensitive Gq/11 family of G proteins, while M2 and M4 bind to the Pertussis toxin-sensitive Gi/G0 family. Nerve cells contain up to four subtypes. Three main functional responses to mAChR stimulation were studied; Post synaptic excitation, post synaptic-inhibition and presynaptic inhibition. Importantly, muscarinic receptors control the time course of evoked ACh release [16,17] and play essential role outside the verves system as well for example in the function of the endothelium, inflammation and urinary bladder [18–21].

Balanced cholinergic signaling thus depends on the concerted expression of multiple receptors, enzymes and transporters. Fig. 1 presents a schematic drawing of the cholinergic synapse and its main components.

Recent findings on peripheral cholinergic signaling renewed the interest in cholinergic-mediated disease processes. Thus, both muscarinic and nicotinic ACh receptors have been identified on lymphocytes isolated from thymus, lymph nodes, spleen and blood [7]. In T-lymphocytes, ACh is synthesized by ChAT and is released upon activation. Furthermore, lymphocytic ACh shows anti-inflammatory properties that inhibit innate immune responses. This mechanism depends on the α7 nicotinic ACh receptor (α7nAChR), which inhibits NF-κB nuclear translocation and suppresses cytokine release by monocytes and macrophages as well. Hence, parasympathetic vagus activation initiates as an anti-inflammatory reflex-like process [11]. Activation of this "cholinergic reflex" has been shown to alleviate inflammatory disease, including endotoxemia and septic peritonitis [11].

Different therapeutic approaches were proposed or are already in use for treating the symptoms of diseases which involve impairments in cholinergic signaling. These diseases span prevalent syndromes which are largely acquired, either with age (e.g., Sjogren's syndrome [22]) and/or due to exposure to environmental factors (e.g., asthma [23]) or surgery (e.g., post-operative apnea [24] or post-operative ileus [25]). Others are autoimmune diseases (such as rheumatoid arthritis [26] or myasthenia gravis [27]) and yet others are inherited (like guttate psoriasis [28]) or drug-induced (post-cocaine poisoning). Most recent examples include small cell lung carcinoma, where ACh was recently proposed to take an active part [29] and chronic allograft vasculopathy, in which impaired cholinergic reaction may cause fatal acute rejection [30]. Compatible with this variety of diseases, the range of proposed and employed therapeutics spans cholinergic agonists and antagonists, biologics (recombinant human cholinesterases) and antisense agents. A relevant example is myasthenia gravis (MG), an acquired autoimmune NMJ disorder in which autoantibodies are directed specifically toward the postsynaptic nAChR. When bound to this receptor, these autoantibodies decrease the number of functional AChRs, leading to a reduction of the postsynaptic depolarization, which results in progressive fatigue of skeletal muscles. The annual incidence of MG is estimated at about 4 to 6 per million, with a prevalence of about 100 per million. At the beginning of the 20th century, approximately 70% of recognized MG patients died, primarily due to respiratory failure or pneumonia. The mortality rate declined dramatically to 15% by 1965 through the development of cholinesterase inhibitors and other therapies, and more recently, antisense suppression of AChE has been developed [27]. For other cholinergic-associated peripheral diseases and proposed cholinergic therapies see Table 1.

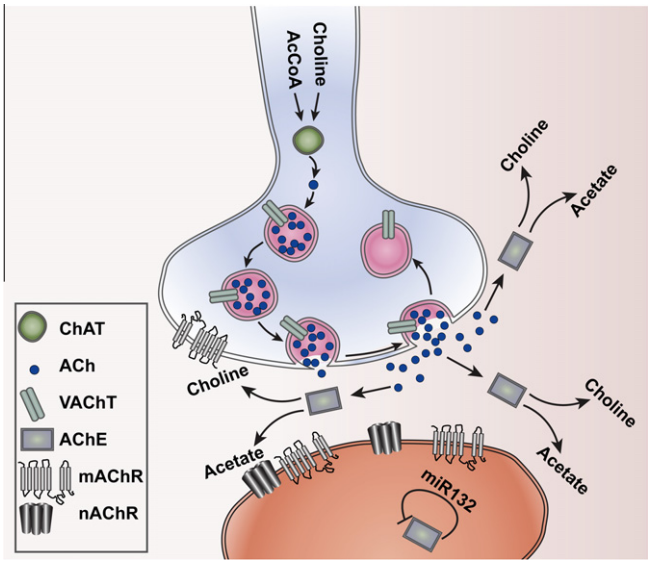

Fig. 1. The cholinergic synapse ChAT catalyses the synthesis of ACh from choline and acetyl-coenzyme A, then vAChT packages it in synaptic vesicles. These vesicles are transported into the synaptic cleft where they can bind nicotinic or muscarinic receptors located on the pre- and postsynaptic membrane. Finally, ACh is hydrolysed in the synaptic cleft by AChE.

2. Measurable cholinergic parameters in body fluids: established uses of available assays

2.1. Quantifying the activities of the acetylcholine hydrolyzing enzymes

The leading method for quantifying cholinesterase activities in body fluids and tissue homogenates is spectrophotometric and