Cholinergic drugs as therapeutic tools in inflammatory diseases: participation of neuronal and non-neuronal cholinergic systems

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Abstract

Acetylcholine (ACh) is synthesized by choline acetyltransferase (ChAT) from acetylcoenzime A and choline. This reaction occurs not only in pre-ganglionic fibers of the autonomic nervous system and post-ganglionic parasympathetic nervous fibers but also in non neuronal cells. This knowledge led to expand the role of ACh as a neurotransmitter and to consider it as a "cytotransmitter" and also to evaluate the existence of a non-neuronal cholinergic system comprising ACh, ChAT, acetylcholinesterase, and the nicotinic and muscarinic ACh receptors, outside the nervous system. This review analyzes the participation of cholinergic system in inflammation and discusses the role of different muscarinic and nicotinic drugs that are being used to treat skin inflammatory disorders, asthma, and chronic obstructive pulmonary disease as well as, intestinal inflammation and systemic inflammatory diseases, among others, to assess the potential application of these compounds as therapeutic tools.

Keywords: acetylcholine- muscarinic receptors-nicotinic receptors-cholinergic drugs- atopic dermatitis- chronic obstructive pulmonary disease- intestinal inflammation- sepsis

Introduction

Organic compounds were formed at the very beginning of the earth in the prebiotic period, as it was demonstrated in the Urey–Miller experiment [1]. It could be probable the acetylcholine (ACh) exists since then, because acetylation of organic molecules like choline, is one of the most common reactions in nature. In addition to its presence in neuronal tissue, there is increasing experimental evidence that ACh is widely expressed in pro- and eukaryotic non-neuronal cells. In fact, the cholinergic system had been created by nature about 2.5 billion years before the first appearance of the neuronal tissue. This can be proved by the fact that ACh is present in bacteria, blue-green algae, yeast, fungi, protozoa and primitive plants [2]. Thus, ACh works as a signaling molecule in non-neuronal cells and tissues, before its neuronal function spans. For these reasons, Wessler et al. have introduced the term "non-neuronal ACh" and "non-neuronal cholinergic system" to underline the presence of ACh in cells independent of neurons or present in organisms free of neuronal tissue during the evolutionary process [3]. In turn, Grando and colleagues introduced the term "universal cytotransmitter", which denotes the involvement of ACh in the regulation of basic and frequently nervous-independent, cell functions like: proliferation, differentiation, organization of the cytoskeleton, local release of mediators (for example: nitric oxide, pro-inflammatory cytokines), locomotion, secretion and ciliary activity [4].

ACh is the main neurotransmitter in the neuronal cholinergic system. This system is conformed by central and peripheral neurons. ACh is synthesized by pre-ganglionic fibers of the sympathetic and parasympathetic autonomic nervous system and by post-ganglionic parasympathetic fibers. The organization of a cholinergic neuron and synapse is well known. In cholinergic neurons the synthesis of ACh occurs within the nerve terminal via choline acetyltransferase (ChAT) enzyme (Fig. 1). ACh is accumulated in vesicles and is released by exocytosis to allow a highly effective neurotransmission. The presence of nicotinic acetylcholine receptors (nAChR) or muscarinic acetylcholine receptors (mAChR) and high acetylcholinesterase (AChE) activity very close to the synapse are needed for the rapid and short lasting action of ACh as neurotransmitter [5].

mAChR belong to the family of G-protein coupled receptors. Five different subtypes have been genetically identified: M_1-M_5 [6]. In airways and lung tissue of most mammals including humans expression of muscarinic M_1, M_2 and M_3 receptors have been described using different techniques such as binding studies with subtype selective ligands, molecular biological and immune-biological techniques, and these findings correlate very well with a large number of functional pharmacological studies. M_1

receptors appear to be expressed particularly in peripheral lung tissue and alveolar wall, but could not be detected in larger airways, skin, intestine and glands. M_2 and M_3 receptors represent the major population of mAChR in smooth muscle fibers, human macrophages from airways and sclera fibroblasts [7, 8]. M_4 and M_5 receptors were described and characterized later than M_1-M_3, and are predominantly expressed in the central nervous system. In urothelium, in endothelial cells and in immune cells that mediate inflammatory reactions, the five subtypes of mAChR have been identified [7].

The coupling of mAChR to their cellular effector systems is mediated via heterotrimeric G-proteins that are composed by one α-, β- and γ-subunit each and are classified virtue to their α-subunit. At the last count, there were 21 Gα subunits encoded by 16 genes, 6 Gβ subunits encoded by 5 genes and 12 Gγ subunits in humans and based on primary sequence homology of the α-subunits, G-proteins have been subdivided into four families: Gαs, Gαi/o, Gαq and Gα12 [9]. Receptor activation results in dissociation of the heterotrimeric G protein into its α-and β/γ-subunits, latter are tightly bound and display one functional unity. Both the α-subunit and the β/γ-subunit are involved in the transduction of muscarinic signals (Fig. 2). Thus, M_1, M_3 and M_5 receptors couple preferentially to G_q proteins, whereas M_2 and M_4 subtypes interact with the Gi/o family of proteins. One important target activated by G_q represents phospholipase C mediating the hydrolysis of phosphatidylinositol 4, 5-bisphosphate to generate inositol 1, 4, 5-triphosphate (IP3) and diacylglycerol (DAG). Gi/o proteins inhibit adenylyl cyclase activity, as well as prolonging potassium channel, non-selective cation channel and transient receptor potential channels opening [6, 10-12]. mAChR have also been demonstrated to regulate a diverse array of signaling intermediates that were not considered as classical. Thus, both Gαi/o- and Gαq/11-coupled subfamily members exert cytoskeletal effects through activation of the small GTPase Rho and downstream effectors have expanded to include phosphoinositide-3 kinases, non-receptor tyrosine kinases and mitogen-activated protein (MAP) kinases [13]. These latter signaling pathways appear to play a major role in the control of cell growth and proliferation as it is shown by Barathi et al. in scleral fibroblasts [14]. These cells respond to muscarinic agonists increasing proliferation via epidermal growth factor receptor, protein kinase C (PKC), proline-rich tyrosine kinase 2 (Pyk-2), B-Raf, Ras, c-Jun N-terminal Kinase (JNK)1/2, and extracellular signal-regulated kinase (ERK)1/2 stimulation. They also observed that carbachol activated p42/44 MAP kinase and Ras in a time-dependent manner [14].

nAChR are a family of ligand-gated, pentameric ion channels. The main function of this receptor family is to transmit ACh signals at neuromuscular junctions and in the central and peripheral nervous systems.

In humans, 16 different nAChR subunits (α1–7, α9–10, β1–4, δ, ε, γ) have been identified [15]. These subunits have the potential to form a large number of homo- and heteropentameric receptors with distinct properties and functions. Among the 16 subunits, only the α1, α7, and α9 subunits bind an antagonist derived from snake venom, α-bungarotoxin. Most neuronal nAChR channels, like muscle nAChR channels, are cation-specific, but do not distinguish readily among cations. Neuronal nAChRs associated channels allow Na^+, K^+ and Ca^{2+} transfer [16].

1. Participation of cholinergic system in inflammation

Inflammation, defined as the development of pain, swelling, erythema and warmth in response to an injury or infection, plays an important role in warding off invasion [17]. The immune system is the first line of defense against invading pathogens and proper functioning of this system is essential for survival. A central feature of the immune response is the production and release of cytokines to orchestrate inflammation. In the vast majority of cases, the innate immune system successfully thwarts pathogenic threats and restores homeostasis.

It has been reported that the non-neuronal cholinergic system is involved in a broader manner in inflammatory situations. Particularly, new observations that show that this system is found in tendinitis, in knee joint synovial tissue, in the gastrointestinal ulcerative mucosa of humans and in a murine model of airway inflammation have been reported [18, 19]. These findings show that the non-neuronal cholinergic system is more widely distributed in the body than previously recognized. The marked existence of this system should be further considered in discussion of the cholinergic influences in disease processes. That includes the processes that occur in diseased tendons and in inflammatory conditions. Moreover it is now known that immune cells (lymphocytes, neutrophils and dendritic cells) that are recruited to inflammatory sites, synthesize ACh [19]. Other cells that usually cooperate with the immune system, like fibroblasts, keratinocytes, endothelial cells, and epithelial cells, also produce ACh. Moreover cells of the immune system contain most components of the cholinergic system, including ChAT, choline transporters, and AChE. In addition, dendritic cells, macrophages , neutrophils and lymphocytes contain both mAChR and nAChR [20-22]. Regarding nAChR, Pavlov et al. discovered that the α7 subunit of these receptors is expressed on macrophages [23]. ACh significantly and concentration-dependently decreases tumor necrosis factor-a (TNF-a) production by endotoxin-stimulated human macrophage cultures via a post-transcriptional mechanism. Using specific muscarinic and nicotinic agonists and antagonists, they demonstrated the importance of an α-bungarotoxin-sensitive receptor in the inhibition of

TNF-a synthesis in vitro by ACh. ACh also is effective in suppressing other endotoxin-inducible pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and IL-8, by a post-transcriptional mechanism [23]. Moreover, previous studies have clearly shown that the activation of nAChR by nicotine suppresses both the adaptive and innate immune responses [24].

However, very little is known about the role of mAChR in the regulation of immune and inflammatory responses. First evidences indicated that carbamilcholine increases the number of leukocytes in the splenic venous blood that is blocked by atropine treatment [25]. Direct addition of ACh to spleen cell cultures enhances the Con A-induced T-cell proliferation [25]. Therefore, activation of mAChR may stimulate the immune system response. However, because ACh can react with cholinergic receptors, it is difficult to ascertain whether the effects reflect its interaction with mAChR and/or nAChR. Interesting results indicated that atropine reduced the antigen-stimulated as well as mitogen-stimulated T-cell responses, suppresses both inflammation (leukocyte accumulation) and tissue injury in response to a sterile abscess, and inhibits both chemokinesis and chemotaxis of leukocytes. Thus, while mAChR agonists stimulate immune/inflammatory responses, mAChR antagonists inhibit these responses [26].

2. Cholinergic drugs in inflammatory process

2.1. Skin inflammatory process

Atopic dermatitis (AD) is characterized by generalized chronic pruritus, inflammation with cellular infiltration, acanthosis and spongiosis. In 1962, a study was published which showed enhanced levels of ACh in biopsy specimens of AD patients, and the author assumed that ACh was originated from sensory nerves or basal epidermal cells [27]. Recent progress in the understanding of the pathophysiology of this skin disease indicates abnormalities in the immune system, such as elevated activation of Langerhans cells and of T and B lymphocytes. In addition, human keratinocytes as well as immune cells infiltrated in the skin at sites of inflammation, synthesize ACh, which acts as a local cell signaling molecule, regulating functions like proliferation, cell adhesion, desmosomal cell contact (barrier function) and glandular activity [28]. It has been reported that ACh induces itching in AD patients and thereby, may be involved in the generation of AD symptoms [29]. Interestingly, the continuous scratching behavior was suppressed by pretreatment with the non-selective mAChR antagonist atropine and by the M_3 receptor antagonist darifenacin, revealing the participation of this receptor in the itch-associated response induced by repeated application of oxazolone in a murine model of AD [30].

The expression of α3, α5, α7, α9, α10, β2 and β4 nAChR in human skin has been reported. In addition, the presence of β1 nAChR mRNA and protein was shown only recently [30]. There seems to be a highly variable expression of the nAChR in the epidermis, especially of the heterooligomeric species of the α3 * -type putative, influencing factors include age, atopic disposition, smoking habits or minimal trauma [31]. Kindt et al. investigated the expression and localization of nAChR α subunits in AD by quantitative reverse transcription-polymerase chain reaction and immunohistochemistry of biopsies from lesional and nonlesional areas of AD skin and of skin biopsies from healthy persons. Data demonstrated the presence of mRNA and protein of the α nAChR subunits not only in keratinocytes but also in mast cells in healthy and AD skin. Expression of the α subunits 3, 7, 9 and 10 was generally reduced in the skin of patients with AD whereas mast cells in AD but not in healthy skin showed α3 and α5 subunit immunoreactivity. AD patients also exhibit increased levels of epidermal ACh. This supports the idea that the cholinergic system is deregulated in AD and that inflammation further modulates individual nAChR subunits. The functional consequence of the down-regulation of nAChR α subunits may be a higher susceptibility of the skin to mediators and factors that lead to eczema or influence the barrier function of the skin. [32].

The skin disease hidradenitis suppurativa (HS) is a chronic inflammatory disabling disease of unknown pathogenesis emerging from the pilosebaceous unit of the intertriginous areas and is hence called acne inversa in the european literature [33]. The pathogenesis of HS has remained elusive, while different triggering factors have been described (tobacco smoking, sweating, and obesity and Staphylococcus aureus infection). In human skin, keratinocytes synthesize and degrade ACh that acts via cholinergic receptors in an autocrine manner and it is also known that ACh acts in a paracrine fashion on the surrounding cells like fibroblasts or melanocytes, modifying their functions [34]. Hana et al. [35] examined the influence of cholinergic drugs in an organotypically cultured epidermis equivalent, from patients with HS and observed that the blockage of mAChR with atropine led to a more pronounced delay in epidermal differentiation and proliferation than blockage of nAChR with mecamylamine. HS often goes undiagnosed for years or is frequently misdiagnosed; prescribed treatments are ineffective, temporary and sometimes even harmful. Atropine (1.2 mg i.v.) was used fifteen years ago in the radical therapy of this illness to block eccrine secretion of skin glands [36]. But this therapy should be revalued in the light of new knowledge [37].

2.2. Asthma and chronic obstructive pulmonary disease

Asthma bronquial, affecting more than 150 million people worldwide, is a major public health problem over the past 20 years. Actually, asthma therapy leads only a limited decrease in death rates [38]. Chronic obstructive pulmonary disease (COPD) is a respiratory illness characterized by chronic obstruction of expiratory flow affecting peripheral airways, associated with chronic inflammation, mucus hypersecretion, gland hyperplasia, in advanced stage, pulmonary remodeling with emphysema and fibrosis [39]. Recent findings indicate that ACh acting on mAChR may contribute to the pathophysiology and pathogenesis of asthma and COPD. In spite that COPD is highly related with smoking, also air pollution, specifically particulate material (i.e. diesel particles) generates inflammation via oxidative stress mechanisms. mAChR in the rat lung are involved in diesel particles-induced neutrophilia [40]. In the latter work authors demonstrated that diesel particles activate a pro-inflammatory vago-vagal reflex which is reduced by atropine; under the conditions of their experiments, nicotinic antagonists did not prevent the acute pro-inflammatory response to diesel particles [40].

Airway smooth muscle expresses muscarinic M_2 and M_3 receptors, roughly in a 4:1 ratio. M_3 receptor couples to G_q protein and is the primary subtype responsible for bronchial and tracheal smooth muscle contraction and glands secretion via calcium mobilization and PKC activation in airway tissues from diverse species, including humans [41]. M_2 receptor on airway smooth muscle preferentially couples to the Go/i protein and function to counteract the β_2 receptor-mediated relaxant pathway by inhibiting the generation and accumulation of cyclic adenosine monophosphate [42]. There are also pre-junctional inhibitory M_2 receptors which activation by released ACh, inhibits ACh liberation in the synapses. Both M_3 and M_2 receptors control airway smooth muscle contraction, because only dual M_2/M_3 mAChR knockout mice had no remaining contractile responses [42]. But in humans, airway smooth muscle contraction occurs largely if not exclusively by M_3 receptors activation [43].

Viral infections are associated with COPD and asthma exacerbations and the presence of virus may affect the structure of the M_2 receptor by the action of neuraminidase, which is contained in the coat of influenza and parainfluenza viruses and is highly expressed by infected tissues. This enzyme cleaves key sialic acid residues from the M_2 receptor surface. As a result, the affinity for agonist at M_2 receptor is decreased and leads to enhanced ACh release after stimulation of parasympathetic nerves, resulting in increased M_3 receptor–mediated contraction of airway muscle [44].

It must be taken in mind that ACh, mAChR and nAChR are not only present in airways smooth muscle, glands and vascular endothelium but also in the immune cells that infiltrate these organs during

inflammatory process. It has been documented that in human inhalation of allergens as well as COPD induce eosinophils and neutrophils influx into the lungs. Eosinophils are responsible of M_2-neuronal mAChR dysfunction in lungs, while ACh promotes leukotriene B_4 production via M_3 receptors in non-septum cells from COPD patients that in turn increased neutrophil recruitment [45]. Gosens et al. demonstrated that secretion of IL-8 and -6 was observed in immortalized airway smooth muscle cells that express muscarinic M_3 receptors and methacholine significantly augmented IL-8 secretion in combination with cigarette smoke extract in a synergistic manner [46]. Although, nicotine is negatively associated with cigarette smoking, addiction, and cardiovascular damage, nicotine also has therapeutic properties and is a promising new treatment for chronic inflammatory disorders. Greene et al. proved the inhibitory capacity of nicotine against TLR2-and TLR4-induced IL-8 production by CFTE29o- airway epithelial cells, pointing to the role of α7-nAChR in these events, and provide data to support the potential use of safe nicotine analogues as anti-inflammatories for cystic fibrosis [47].

Chronic inflammation in asthma and COPD drive pathological structural remodeling of the airways. Profita et al. found and increment in ChAT, mAChR, ERK1/2 and nuclear factor (NF) κB expression in lung fibroblasts from patients with COPD and smokers. ACh stimulation increased fibroblast proliferation in these patients and anti-cholinergic drugs, including tiotropium, might prevent these events [48]. Using tiotropium bromide, ACh was recently identified as playing a major regulatory role in airway smooth muscle remodeling in a guinea pig model of ongoing allergic asthma [49]. In this paper, authors demonstrate that tiotropium is similarly effective to the glucocorticosteroid, budesonide, in inhibiting several aspects of airway remodeling, and provide further evidence that the beneficial effects of tiotropium bromide might exceed those of bronchodilation [49]. In addition, it was demonstrated that tiotropium bromide can inhibit Th_2 cytokine production and airway inflammation, and thus may reduce airway remodeling and airway hyperresponsiveness in a murine model of asthma [50].

The advent of modern inhaled anti-cholinergic compounds to treat airways pathologies depended on the ability to prevent systemic absorption of these molecules while retaining potent anti-cholinergic activity. The results were highly charged quaternary ammonium salts that were poorly absorbed across membranes and, thus, had low oral and systemic bioavailability and low blood–brain barrier penetration but were still highly potent antagonists at mAChR in the airways. It has been recently reported that formoterol and ipratropium bromide partially protect the lungs against inflammation by reducing neutrophilic infiltration. This protective effect is associated with reduced metalloproteinase-9 activity known to play an important

pro-inflammatory role in acute inflammatory process [51]. In spite of this, tiotropium bromide is considered a true second-generation quaternary anti-cholinergic because this molecule is an order of magnitude more potent than ipratropium bromide and has a long half-life at M_3 mAChR ($t_{1/2}$:35 h) [52]. In addition to its muscarinic antagonistic actions at airways level, tiotropium suppresses the ACh-induced release of chemotactic agents, mainly leukotriene B_4 from primary human macrophages as well as myeloid cell and epithelial cell lines via the blockade of mAChR. This mechanism may explain the anti-inflammatory action of tiotropium which may contribute to reduce exacerbation observed in animal models and clinical studies [52, 53].

2.3. Intestinal inflammation

Intestinal inflammation impairs agonist induced contraction of smooth muscle, but the cellular basis for this remains to be fully elucidated. Furthermore, the diversity of neurotransmitters and other contractile agonists present within the inflamed intestine adds further complexity to the understanding of altered contractility. However, much of the current literature has focused on the contractile response to ACh as the primary neurotransmitter of the gut. The essential role of mAChR in cholinergic contraction of colon smooth muscle cells (CSMC) has been well established. Under control conditions, ACh-induced contraction is mediated solely through the M_3 receptor but during inflammation, the M_2 receptor pathway becomes functionally active [54]. No studies have examined smooth muscle cell receptor usage following resolution of inflammation. Previous studies demonstrated that inflammation caused alterations to the use of mAChR at the level of the smooth muscle cell [55]. Wells et al. showed that ACh-induced contraction was predominantly mediated by M_3 receptors under control conditions, which is consistent with findings in other systems [56]. Acute inflammation causes a functional switch in the role of the mAChR leading to a decrease in CSMC contraction with paralleled modulation of mAChR utilization. They found a parallel decrease in the functional utilization of the M_3 receptor in the inflamed colon, as the selective antagonists, 4-DAMP and pF-HSD became less effective in blocking contraction [56]. As M_3 receptor utilization decreased, there was a substantial increase in the dependency on M_2 receptor in ACh mediated contraction, as methoctramine displayed increased effectiveness on day 4 of colitis [56].

Irritable bowel syndrome (IBS) is a functional disorder, which has recently been linked to immune activation. The pro-inflammatory cytokine profile in IBS is driven by the cholinergic system and determined if the responses are mediated by mAChR. In a recently performed clinical study, it was demonstrated that patients with IBS present higher levels of the pro-inflammatory cytokines, IL-6 and IL-

8 than controls in blood samples. Particularly, patients alone exhibit an exaggerated mAChR-mediated IL-6 response that was reduced by procyclidine a muscarinic antagonist with central action [57]. Intestinal colonic smooth muscle cells express the muscarinic receptors M_2 and M_3 in a ratio of approximately 4:1, respectively. It has been demonstrated that ACh induced contraction of rat colonic smooth muscle cells was mediated predominantly by M_3 receptors as selective M_3 antagonists blocked most of the response. If M3 antagonists are able to induce anti-inflammatory actions besides their antispasmodic actions, as it is clearly stated in COPD,needs further experimental evidences.

The cholinergic nervous system also attenuates the production of pro-inflammatory cytokines and inhibits inflammatory processes. One of the gastrointestinal (GI) disorders in which vagus nerve stimulation has been shown to ameliorate disease is postoperative ileus (POI) [58]. POI is a commonly occurring postoperative complication that results from manipulation of the bowel during abdominal surgery, and is characterized by a transient hypomotility of the GI tract. In rodent models of POI, intestinal manipulation leads to leukocyte influx into the muscularis externa, resulting in delayed gastric emptying and impaired small intestinal transit. Electrical stimulation of the vagus nerve can reduce recruitment of neutrophils and restore gastric emptying in mice. The anti-inflammatory effect attained with electrical vagus nerve stimulation can be mimicked by AR-R17779, which specifically targets the nAChR α7 subunit. In experimental models of acute colitis, the vagus nerve seems to possess regulatory properties in inflammatory responses. Several studies show that nicotine administration attenuates disease in colitis models, although fairly high doses of nicotine are required.

On the other hand, in infectious models of microbial peritonitis, vagus nerve activation seemingly acts counteractive; it impairs bacterial clearance and increases mortality. It is originally indicated that the key mediator of the cholinergic anti-inflammatory pathway, ACh, inhibits cytokine release directly via the α7 nAChR expressed on macrophages. However, more recent data also point towards the vagus nerve as an indirect modulator of innate inflammatory processes, exerting its anti-inflammatory effects via postganglionic modulation of immune cells (macrophages, monocytes and mast cells) in primary immune organs [58].

2.4. Systemic inflammation

Sepsis is the most common complication in patients admitted to surgical intensive care units. It is usually initiated by microbial pathogens. The most commonly accepted explanation for the development of sepsis involves an overwhelming inflammatory response [59]. Lipopolysaccharide (LPS), a cell wall component

of Gram-negative bacteria, mimics several of the symptoms observed in sepsis, including the rapid release of inflammatory mediators such as TNF-a, IL-1β and gamma interferon. In previous studies, Fuentes et al. showed that the administration of general anesthetics resulted in a modification of the inflammatory process and improved survival after endotoxic shock [60]. The modulation of the inflammatory response by these drugs, in particular isoflurane, was not mediated through the vagus nerve. The same group observed an inhibitory effect on the LPS-induced production of TNF-a after treatment with atropine [61]. This antagonist is commonly used as an adjuvant in general anesthesia to block responses to vagal reflexes induced during surgical manipulation of visceral organs. Previous observations of the effect of atropine on the inflammatory process were enriched by results that demonstrated a significant improvement in the survival of endotoxic shock following treatment with atropine. Herewith, previous results have shown that atropine treatment resulted in attenuation of TNF-a levels and elevation of IL-10 production [60].

Whilst muscarinic signaling in peripheral organs is not required for vagus nerve control of inflammation, central muscarinic transmission is important in attenuating inflammatory responses. Intracerebroventricular administration of muscarine or the M_1 muscarinic receptor agonist McN-A-343 attenuated serum TNF-a levels in a rat model of endotoxemia [61]. The same authors demonstrated that intracerebroventricular administration of methoctramine, a M_2 receptor antagonist that enhances ACh release in brain, attenuated systemic TNF-a in endotoxemic rats and augmented vagus nerve activity [62]. The promotion of nAChR activation has similar beneficial effects in endotoxemia models as mAChR blockade alone. Sun et al. [63] documented that in mice with experimental endotoxemia, combined administration of anisodamine, muscarinic antagonist and neostigmine, an inhibitor of AChE, significantly increased the survival rate and decreased the serum levels of inflammatory cytokines, as compared to those produced by either drug alone. The anti-shock effect of combined anisodamine and neostigmine was abolished in α7 nAChR knockout mice. On the other hand, intravenous injection of the combined anisodamine and neostigmine, or the selective α7 nAChR agonist PNU282987 exerted similar anti-shock effects in dogs with hemorrhagic shock. The results demonstrate that combined administration of anisodamine and neostigmine produces significant anti-shock effects, which involves activation of α7 nAChRs.

Conclusions

Inflammation may up regulate ACh synthesis. In addition it is necessary to analyze the changes of other main components of the cholinergic (neuronal or non-neuronal) system such as: mAChR, nAChR ChAT, AChE in models of acute (systemic) and chronic inflammation in different organs (skin, lungs, intestine) in human diseases. Expression and function of both, mAChR and nAChR, can be modified by different mediators, including inflammatory cytokines and also autoantibodies. The interpretation of these complex net of interactions between central and peripheral cholinergic system or neuronal and non-neuronal cholinergic systems and inflammatory milieu will optimize the use of muscarinic antagonists and/or nicotinic agonists in inflammatory process (Table 1). The use of cholinergic drugs in the treatment of inflammation might be associated with new relevant pharmacological approach in acute or chronic inflammatory processes.

List of abbreviations

Ach= Acetylcholine

AChE= Acetylcholinesterase

AD= Atopic dermatitis

ChAT= Choline acetyltransferase

COPD= Chronic

CSMC= Colon smooth muscle cells

DAG= Diacylglycerol

ERK= Extracellular signal-regulated kinase

HS= Hidradenitis suppurativa

IBS= Irritable bowel syndrome

IL= Interleukin

IP3= Inositol 1, 4, 5-triphosphate

JNK= c-Jun N-terminal Kinase

LPS= Lipopolysaccharide

mAChR= Muscarinic acetylcholine receptors

MAPK= Mitogen-activated protein kinase

nAChR= Nicotinic acetylcholine receptors

NFκB= Nuclear factor kappa B

PKC= Protein kinase C

POI= Postoperative ileus phosphatidy

Pyk-2= Proline-rich tyrosine kinase 2

TNF-a= Tumor necrosis factor-a

CONFLICT OF INTEREST

None declared.

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Legend of Figures

Figure 1. Biosynthesis of acetylcholine. ChAT: choline acetyltransferase. CoA: coenzyme A.

Figure 2. Subtypes of cholinergic receptors, transduction and effects. mAChR: muscarinic acetylcholine receptors; nAChR: nicotinic acetylcholine receptors; ACh: acetylcholine; PLC: phospholipase C; IP3: inositol triphosphate; DAG: diacylglycerol; PKC: protein kinase C; AC: adenilyl cyclase; cAMP: cyclic adenosine monophosphate; IP3K: inositol triphosphate kinase; JNK: janus kinase MAPK: mitogen activated protein kinase; ERK: extracellular kinase; NF-κB: nuclear transcription factor-κB; iNOS: inducible nitric oxide synthase; TNF-a: tumor necrosis factor alpha; IL: interleukin; HMGB1: high mobility group box 1.