REVIEW

α7 Nicotinic acetylcholine receptor-mediated anti-inflammatory actions modulate brain functions

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The interaction between the immune system and the central nervous system is largely unknown and under intense scrutiny by the biomedical community. Research results during the last decades have identified important two-way communication processes between these two systems, mediated by the cytokine network as well as by "classical" neurotransmitters. The dogma of a separate functioning of the two systems has been conclusively challenged with the discovery that not only neurotransmitters affect immune system function, but also inflammation affects neuronal cells through the same "cytokine network" that connects the different components of the immune system. The "classical" transmitter acetylcholine is an important modulator of both neuronal and immune function through both muscarinic and nicotinic receptors. Among the latter, α7 nicotinic acetylcholine receptors (nAChRs) possess the peculiar property of being expressed in immune cells as well as in neurons. While the modulation of neuronal activity by direct activation of α7 nAChRs is relatively well described, the hypothesis that α7 nAChRs may influence neuronal behavior indirectly, through inhibition of inflammation, is a relatively new concept. This review aims to summarize the evidence that activation of α7 nAChRs may influence brain function not only by direct action on certain neuronal pathways, but also by reducing inflammation (central and/or peripheral), decreasing the levels of circulating cytokines and consequently, their influence on neuronal activity.

Keywords: Acetylcholine; alpha7 nicotinic receptors; inflammation; synapses; glia

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Introduction

The mammalian immune system as a ubiquitous organ regulator

Important functions in any living organism are the defense from microbiological insult or from the development of cancerous cells triggered by exogenous or endogenous stimulus. Mammals carry out these functions through the immune system, with a set of organs and cells interacting with each other, by means of a highly coordinated activity aimed to identify, react, and eventually allow healing of each

and every part of the body that may undergo such insults. The potential for a swift, chemically and anatomically specific, sometimes systemic action of the immune system is associated with a high energy demand in order to face equally aggressive pathogen, toxin, or neoplastic invasion. Such need for high sensitivity and a varying range of metabolic consumption is controlled by a highly sophisticated and only partly understood "accelerator-brake" system, using the cytokine network. This network is composed by a complex set of interconnected molecules whose biochemical interactions involve not only cells and organs of the immune system, but also parts of the central, autonomic, and endocrine nervous system [1]. While a failure in the "accelerator" may obviously lead the organism to succumb due to pathogen, toxin, or neoplastic damage, failure in the "brake" part of the immune system control may, less obviously but equally importantly, be associated with powerful self-induced over-active harmful reactions, such as allergies or auto-immune diseases [2].

For historical reasons, the immune system response has been divided into adaptive system response, characterized by a slow antigen-specific activity, and an innate system response, associated with fast and less specific antigen-triggered responses. In the actuality, adaptive and innate responses share a wide range of mechanisms as well as of biochemical and cellular substrates, of which inflammation is an important component [1].

Immune response in the central nervous system

Like all parts of the mammalian organism, the CNS is also subject to immune control. The brain is physically and biochemically separated from the rest of the body by the blood brain barrier (BBB), a cellular layer of glia and other specialized cells present in the endothelium of the brain vasculature that limits and controls chemical and cellular exchange between the CNS and the periphery [3]. In normal conditions, the access of immune cells of the blood and of the lymphatic system is also limited by the BBB, determining different immune responses in the CNS vs. the periphery, property that is often referred to as "immunoprivileged" status of the CNS. Trauma, stroke, stress, and disease in general, may compromise the physical and/or biochemical function of the BBB, occasionally allowing the permeation of macromolecules as well as immune cells whose crossing to the brain compartment from the periphery is normally banned. In some instances, even in the absence of occasional or pathology-driven changes in permeability of the BBB an inflammatory response in the brain may be induced by activation of non-neuronal components of the CNS, particularly microglia, the resident macrophages of the brain, which express α7 nicotinic acetylcholine receptors (nAChRs).

This receptor subtype plays an important role in the shut-down process of the inflammatory response, once the physiological need for inflammation subsides [4].

Link between immune activation, neuropsychiatric diseases, and nicotinic receptors

A line of work relating behavioral changes detected in clinical settings as well as in animal models suggests that elevated levels of immune-related molecules are associated with psychiatric diseases [5, 6] and with the decline of psychological performance [7]. A causal role for inflammation has been proposed for neuropsychiatric conditions including depression and anxiety disorders, as well as neurodevelopmental conditions such as schizophrenia and autism spectrum disorders (ASDs) [8, 9]. The model of Maternal Immune Activation (MIA), consisting in provoking aseptic inflammation with the injection of lipopolysaccharide (LPS) or viral-like double strand RNA (poly I::C) in the pregnant experimental animal is being particularly useful in the investigation of the link between type, duration, and severity of the prenatal inflammation on one hand, and in the nature of the postnatal damage on the other [10].

Several lines of evidence relate inflammation with altered brain function in acute trauma and anesthesia: a decrease of mental capabilities is the cognitive decline associated with major surgery (post-operative cognitive decline) [11]. Major surgeries such as open-heart surgery, orthopedic surgery, and organ transplants, are very frequently followed by depression [12, 13], which at times takes a lot longer to recover than the physical recovery from the surgery per se. Additional support to the link between inflammation and CNS function comes from clinical and animal studies showing that stress alters brain levels of pro-inflammatory cytokines in a pattern-specific manner [14-16], and that alterations in the function of microglia may play a key role in an inflammatory component of neuropsychiatric diseases [17].

So far, no relationship has been determined between the findings exposed above with a role for α7 nAChR dysregulation, as proposed for a number of psychiatric pathologies including schizophrenia [18, 19], ADHD [20], and autism [21]. In fact, the presence of brain nAChRs does not rule out other avenues of nicotinic modulation of neuronal function and macroscopic behavior by the same receptors.

The observations above open the door to unexplored interpretations of the ill-understood etiology of a wealth of neuropsychiatric conditions like schizophrenia, autism, and depression, whose current treatments offer yet a limited way out to the psychiatric patient, being not only scarcely effective, but having severe side effects. Among them, we

can quote changes of personality, inertia, loss of cognitive and mnestic abilities, pharmaco-dependence caused by prolonged use of serotonin (SSRIs) and/or norepinephrine (NSRIs) selective reuptake inhibitors [22], or by GABA$_A$R enhancers like benzodiazepines or barbiturates [23], or the deleterious cognitive effects caused by electroconvulsive therapy [24]. The importance of α7 nAChR-mediated anti-inflammatory responses in the modulation of behavior is prompted by the observation that nicotine administration decreases the extent of LPS-induced sickness behavior [25]. In the following paragraph we will briefly review the relation between a few neuropsychiatric conditions, inflammation, and α7 nAChRs.

Schizophrenia

A cholinergic theory of schizophrenia was proposed by several authors in the '70s involving a role of muscarinic AChRs (mAChRs) [26], and was further refined in the '90s by integrating a functional alteration in the cholinergic system [27]. The cholinergic theory of schizophrenia was substantiated by observations such as that the density of both nAChR and mAChR types is altered in brain areas whose activity is abnormal in psychotic schizophrenia [28, 29].

A further link between schizophrenia and cholinergic function, specifically nAChRs, is based on the observed higher number of smokers among schizophrenic patients compared to the general population, considered by many clinicians a form of self-medication [30]. Alternatively or additionally to an increased sensitivity to the central -addictive- effects of nicotine, a nicotine-induced anti-inflammatory systemic reduction of an abnormally elevated level of basal inflammation may be a factor in the high percentage of smokers among schizophrenic patients [31, 32]. The same observation would also supply an alternative interpretation to the recurrent presence in schizophrenic patients of the deletion of the region 15q13.3, which, among other genes, encodes for the α7 subunit, and consequently, producing a mutated α7 subunit [33]. An animal model suggests that such deletion decreases the number and function of fast-spiking GABAergic interneurons containing the Ca^{2+}-binding protein parvalbumin (PV+) in the hippocampus and in the cortex [34], which is possibly the most reproduced alteration in autoptic schizophrenic brain [35]. The same group has identified a further connection between α7 nAChRs and schizophrenia, showing that α7-null mice display a deficit in N-methyl-D-aspartate receptor (NMDAR) expression, which is associated with NMDAR hypofunction [36, 37], a well-recognized cause of acute as well as chronic psychosis. The observed characteristics regarding the GABAergic interneuron and NMDAR deficit associated with alterations of α7 nAChR function suggest that the link between α7 nAChRs and schizophrenic psychosis may be primary in the etiology of the condition.

On the basis of previous observations it is reasonable to hypothesize that a reduced expression of α7 nAChRs might decrease the physiologic activation of anti-inflammatory processes in the brain, affecting prevalently more vulnerable neuronal types, including fast-spiking PV+ GABAergic interneurons, causing the appearance of psychotic symptoms in individuals subject to strong or prolonged inflammatory stressors.

Autism

Autistic Spectrum Disorders (ASDs) are a series of neurodevelopmental conditions associated with stereotyped interests and behaviors, and diminished social interaction and communication [38]. Importantly, ASD patients present a recurrent micro-deletion of chromosome 15q13.3, which encodes for the α7 subunit [39, 40], together with alterations in distribution and function of other nAChR subunits [21, 41, 42]. The observation that non-α7 nAChRs are downregulated [43] while α7 nAChRs are up-regulated in the autistic brain [41], together with the finding that α7 nAChRs regulate the development of sensorial neural pathways from the brainstem to the neocortex [44, 45], has given rise to a "nicotinic" hypothesis of ASD. This hypothesis poses that an early -possible fetal- alteration in the nicotinic system of the mammal would impair the correct neural development of sensory pathways, leading, in turn, to a gross malfunction of "higher" circuits, including those regulating social behavior and communication. In line with these findings, the use of nicotinic ligands has been proposed in the pharmacologic therapy for ASD [46].

Alternative hypotheses are fostered by consideration of a series of observations that link the onset of at least some type of ASD with immune activation [47, 48]. For instance, ASD patients display a high incidence of gastro-intestinal problems [49, 50], supporting the hypothesis that important alterations of immune function are possibly of primary nature [51]. In addition, numerous studies have identified critical time windows of fetal and perinatal development in which maternal immune hyper-activation (MIA) may cause ASD-like conditions in humans [52] as well as in animal models [53]. The influence of the developing immune system in the etiology of autism is very poorly understood [54, 55], and warrants further investigation.

Depression

Clinical evidence in neoplastic disease shows that circulating pro-inflammatory cytokines induce depressive

symptoms [56, 57]. These data are corroborated by animal model studies in which depressive-like behaviors are observed upon pro-inflammatory cytokine administration [58]. These results have been related to sickness-behavior also associated with increased pro-inflammatory cytokine levels [59].

A leading theory for the genesis of depression is the convergence of a genetic component with a series of stressful life-events, leading to the decrease in the effectiveness of the inhibitory synaptic system in the limbic cortex [60] caused by a sustained elevation in the levels of the pro-inflammatory cytokine IL-6 [58, 61]. These and other observations opened the way to a new hypothesis postulating a role for inflammation in the etiology of depression, with the corollary that the use of any ligand that decreases the release of pro-inflammatory cytokines, including α7 nAChR agonists, may be beneficial for the treatment of this condition [62]. To our knowledge, no attempts have been made so far to use nAChR ligands with such anti-inflammatory activity to ameliorate the symptoms of depression.

Differential role of acetylcholine between the CNS and the immune system

Acetylcholine (ACh) is a biogenic amine largely available in the living world, acting through mAChRs and nAChRs [63]. Its effects have been first described in the pioneering work on the autonomic system by John Langley, and then hypothesized to affect the cardiac muscle (Otto Leuwi's "vagustoff"), before being identified as a neurotransmitter in 1913 by Henry Dale and co-workers [64, 65]. Since then, ACh receptors have been identified in a large variety of neuronal types, including motoneurons [66], autonomic ganglia [67], and brain neurons [68], as well as non-neuronal cells [63, 69, 70].

Mechanisms of action of muscarinic and nicotinic receptors

mAChRs are represented by 5 families of GTP-binding 7-membrane spanning membrane proteins which, upon binding with muscarinic agonists, initiate a second messenger cascade leading to either a decrease in the activity of adenylyl cyclase and subsequent levels of cAMP or the activation of phospholipase C and the corresponding intracellular cascade (production of DAG and IP_3, and opening of intracellular Ca^{2+} stores) [71]. Non-neuronal mAChRs are found in the skin, secretory glands, the lungs, in the digestive tract, in the urinary system, in the reproductive system, the cardiac muscle, and smooth muscle [72], as well as immune cells [70]. The typical affinity range of acetylcholine for mAChRs is in the tens nanomolar range [73], a relatively low concentration that can be comfortably reached in the extrasynaptic space, supporting the hypothesis of a corresponding paracrine large distance range termed "volume transmission" [71]. In many cell types, including neurons, mAChR activation induces a plethora of cellular effects, whose best described one is perhaps the closure of K^+ channels [74, 75], generally producing an excitatory effect on neurons. On the other hand, nAChRs are represented by a family of pentameric membrane channels, typically formed by different subunits (i.e. heteromeric receptors), including α subunits (α1-α10), β (β1-β4), γ, δ, and ε subunits [76, 77]. The best characterized heteromeric receptors are the muscle nAChRs, including the embryonic (α1β1γδ) and adult (α1β1εδ) forms. There is also evidence that only one subunit (i.e., homomeric receptors) can form functional channels (e.g., α7, α8, and α9). The ACh binding sites have been identified within the interface of two subunits, α/β in heteromeric nAChRs, or α/α in homomeric nAChRs.

Similar to mAChRs, nAChRs also may respond to volume transmission although micromolar agonist concentrations are necessary to gate these receptors [78]. Some of the nAChRs, and specially α7 nAChRs, possess a substantial permeability to Ca^{2+} [79], putting them in the list of molecular candidates modulating neuronal plasticity. The lower affinity of nAChRs for the natural agonist ACh -compared to mAChRs- suggests their involvement in negative feed-back mechanisms associated with increasing levels of ACh, possibly through presynaptic modulation.

The next two sections will describe the function of mammalian α7 nAChRs in neurons [80] and immune cells [81].

Direct regulation of central nervous function by neuronal nicotinic receptors

In vivo as well as in vitro studies suggest that α7 nAChRs are involved in nearly all kinds of brain functions, including cognitive [82], emotional [83], mnestic [84], perceptual [85, 86], and motor [87].

Earlier immunohistochemical data have suggested that ACh regulates brain function through a number of different nAChR subtypes [88, 89]. In the rodent hippocampus, the most abundant heteromeric nAChRs are the α4β2 and α3β4 subtypes, whereas α7 nAChRs have been found in the cortex both in glutamatergic [90] and GABAergic [34] neurons as well as in neurons from several other brain areas [89, 91-95]. We will not analyze in detail the extensive issue of the direct regulation of the CNS by nAChRs, which is not central to our discussion. We refer the interested reader to two recent reviews on the topic [82, 84], as well as on other chapters of the present issue.
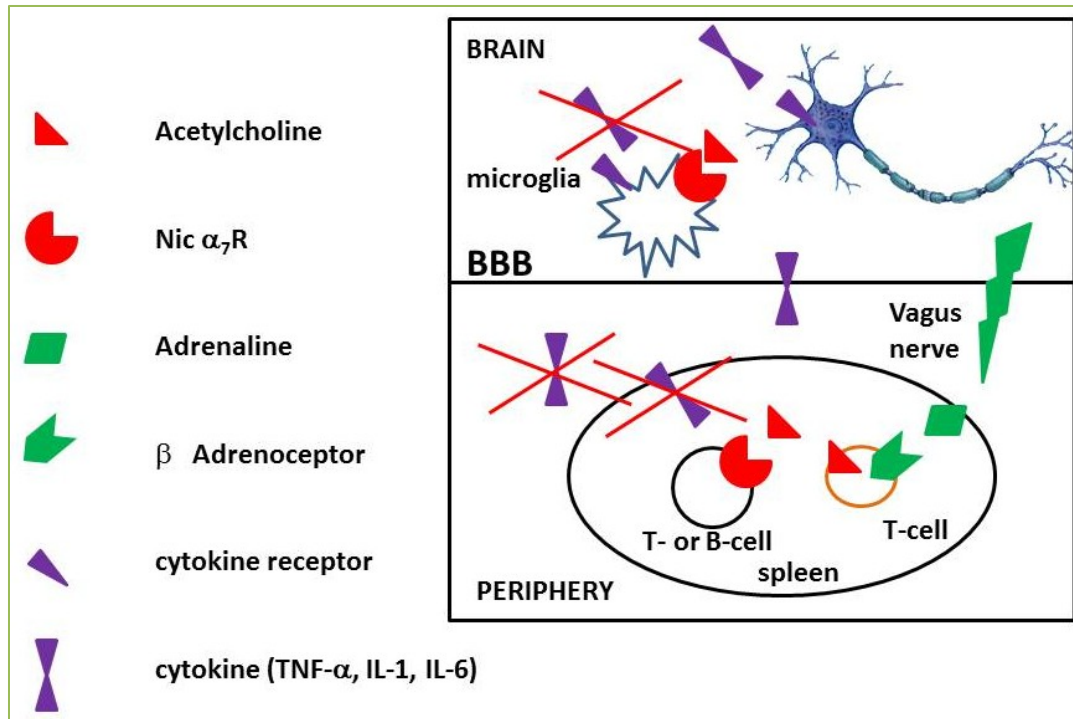
Figure 1. α7 nAChR activation produces immune effects in the CNS. In pathologic conditions, stressors increase inflammation through activation of immune cells, eliciting the release of cytokines which eventually alter neural function either directly, penetrating the BBB through carriers or because of its decreased effectiveness, or indirectly, by activating glial cells. The activation of the descending branch of the mixed sympathetic/parasympathetic branch of the autonomic system releases epinephrine which activates an ACh-synthesizing subtype of T-cells in the spleen (and in other immune organs). The release of acetylcholine by these specialized T-cells, in turn, activates α7 nAChRs located either in the outer mitochondrial membrane (see Fig. 2) or internal compartment of T-, B- or dendritic-cells, inhibiting their activation. Inhibited lymphocytes decrease their cytokine production, restoring or recovering normal neuronal function.

Our next sections will consider the possibility that ACh as well as endogenous and exogenous nicotinic ligands, particularly selective for the α7 subtype, modulate behavior in an indirect manner, more specifically by modulating neurons through altering immune function.

Cholinergic regulation of immune function

Both mAChRs and nAChRs modulate immune response [96]. A distinctive feature of the cholinergic modulation of immune cells is the opposite direction of the effects mediated by mAChRs vs. nAChRs. In fact, while large evidence exists on the immune-suppressor and anti-inflammatory effects elicited by nAChRs, mAChR activation produces solid immune activation of both the innate and the adaptive immune systems [97, 98]. Most of the anti-inflammatory nicotinic mediated responses have been associated with the activation of α7 nAChRs [97].

Interestingly, the α7 nAChR function can be modulated by endogenous peptides [99]. Two endogenously occurring modulators of nAChRs named SLURP-1 and SLURP-2

(secreted mammalian Ly-6/uroquinase plasminogen activated receptor related protein 1 and 2, respectively) have been previously identified in many organs and cell types, including immune cells [100, 101]. In particular, it has been proposed that SLURPs are produced after the activation of a regulatory subtype of T-cell that concur to terminate peripheral inflammation [102, 103]. SLURP-1 and -2 are considered autocrine and paracrine molecules that regulate keratinocyte proliferation, apoptosis, and cell differentiation (reviewed in [77]). In particular, SLURP-1 is considered an endogenous positive allosteric modulator (PAM) of α7 nAChRs (i.e., potentiates the activity of α7 nAChRs without activating the receptor *per se*; reviewed in [77]), whereas SLURP-2 is contemplated as a competitive antagonist of α3-containing nAChRs. Mutations in the SLURP-1 gene are involved in the development of the Mal de Meleda (i.e., a rare autosomal recessive condition characterized by inflammation and hyperkeratosis of the palms and soles), which is observed in smokers with higher frequency than that in nonsmokers, whereas SLURP-2 is found to be upregulated in psoriatic nonlesional skin, and increases the number of

keratinocytes in culture and their resistance to apoptosis (reviewed in [77]).

Immune system-mediated effects of α7 nAChRs in the CNS

Given their anatomical, biochemical, and functional pleiotropy, it is not far-fetched to hypothesize that the activation of α7 nAChRs drastically modulates animal behavior, not just through direct neuronal actions [89], but also indirectly, by altering neuronal function through changes in the checks and balances of the cytokine network, acting on microglia and astrocytes [97]. This latter effect has the potential key factor tipping the balance between neurotoxicity and neuroprotection, in the apparently contradictory interplay between promoting neuronal degeneration on one hand as well as neuronal survival and growth on the other [104, 105].

α7 nAChR activation decreases inflammation

Abundant clinical evidence as well as works with animal models supplied evidence that the activation of α7 nAChRs contributes to the subsiding inflammation. For instance, large ischemic damage is caused by microglia activation following, for instance cardiopulmonary episodes, and the activation of the anti-inflammatory reflex through α7 nAChRs contributes to a substantial reduction of such inflammatory damage [106]. Ischemic damage appears to negatively regulate the expression of microglial α7 nAChRs, effectively inhibiting the negative feedback induced by the anti-inflammatory reflex in the decrease of inflammation [107].

These results are in agreement with another study showing that the activation of α7 nAChRs increases survival rates following LPS administration and sepsis [108], possibly by reducing the release of the pro-inflammatory cytokine tumor necrosis factor α (TNF-α), as shown for a zymosan-induced peritonitis from macrophages of the gastrointestinal tract [109]. An α7 nAChR-mediated decrease in inflammation could also be activated following the increase in the concentration of ACh following the use of tacrine or other acetylcholinersterase (AChE) inhibitors in the treatment of Alzheimer Disease (AD) [110].

α7 nAChR activation mediates the anti-inflammatory reflex

The "anti-inflammatory reflex" is an important part of the regulation of the "brake" system for the innate response. This reflex, consisting in the attenuation of peripheral inflammation following activation of the descending branch of the vagal nerve, was discovered by Tracey's group in the past decade [111] (Fig. 1). The "anti-inflammatory reflex", which has been further studied and described by several other groups as well [112, 113], modulates immune function through an autonomic adrenergic- and cholinergic-mediated process associated with the activation of the motor end of the vagal nerve [114], resulting in a reduction of inflammation as well as of immune activation in general, mediated by activation of α7 nAChRs [115].

In the presence of an external microbiological aggression or other external or internal challenge, stressors increase inflammation through activation of immune cells, eliciting the release of cytokines which eventually alter neural function either directly, penetrating the BBB through carriers or because of its decreased effectiveness, or indirectly, by activating glial cells. Under these conditions, the activation of the descending branch of the mixed sympathetic/parasympathetic branch of the autonomic system induces the release of epinephrine, which, in turn, activates an ACh-synthesizing subtype of T-cells in the spleen (and in other immune organs). The release of ACh by these specialized T-cells eventually activates α7 nAChRs located either in the outer mitochondrial membrane (see Fig. 2) or internal compartment of T-, B- or dendritic-cells, inhibiting their function by preventing activation of the inflammasomes, caspase-associated cytosolic complexes of protein whose activation yields the production of pro-inflammatory cytokines. Inhibited lymphocytes decrease their cytokine production, restoring or recovering normal neuronal function.

It is important to mention that ACh is not the only endogenous compound involved in the control of inflammation through the anti-inflammatory reflex. In fact, in spite of being the vagal nerve mostly associated with cholinergic mediated parasympathetic activity, the "anti-inflammatory" activity of the vagal nerve is mediated by norepinephrine (NE), mostly released onto the spleen. The activation of adrenoceptors expressed in splenic T-lymphocytes triggers the synthesis and release of ACh, which in turn activates α7 nAChRs, reducing its pro-inflammatory activity [116, 117]. In addition to this process, non-α7 nAChR-dependent mechanisms have also been found in the cholinergic-induced decrease of inflammation [118].

Is an anti-inflammatory reflex present also in the brain?

While the existence and function of a peripheral anti-inflammatory reflex has been solidly proven and understood [92, 94, 105], only more recent studies are investigating the potential of a similar modulatory mechanism for CNS inflammation [4, 119]. It is worth mentioning in this context that T-cell receptors have recently
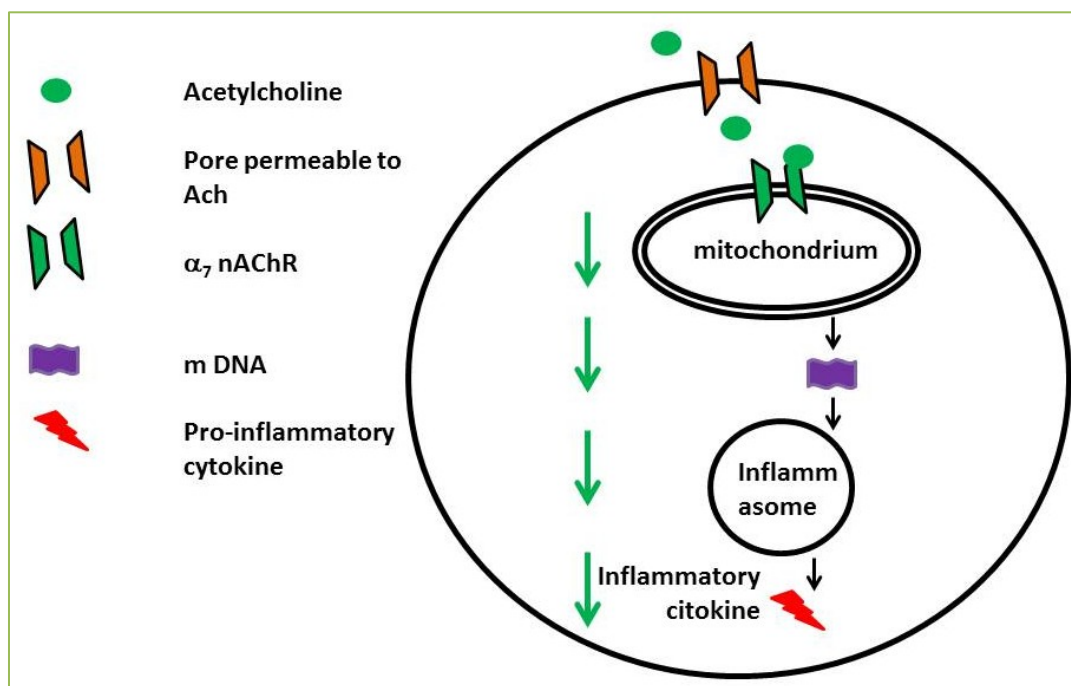
Figure 2. Mitochondrial α7 nAChRs decrease inflammation. The discovery of ACh-permeable pores in the cell membrane, together with the presence of α7 nAChRs in the outer mitochondrial membrane has led to the discovery that ACh decreases inflammatory cell activity by reducing the release of pro-inflammatory mitochondrial DNA by inhibiting the activity of cellular immune cell inflammasomes, cytosolic complexes of protein containing several caspases whose activation yields the production of pro-inflammatory cytokines.

been identified in cortical neurons, where their activation reduces α7 nAChR mediated currents, decreasing neuronal excitation [120]. The pathophysiological meaning of this finding is still unknown.

Several lines of evidence suggest that an anti-inflammatory reflex is present and effective within the CNS, largely mediated by activation of α7 nAChRs from microglia [4, 121]. The presence of a duplicated α7 (dup-α7) gene in the CNS, as well as in other tissues [122], is intriguing. The dup-α7 gene has been suggested to regulate the assembly and possibly reduce the transport of functional α7 nAChRs from the cytoplasmic compartment where they are synthesized and assembled to the membrane compartment where they contribute to membrane depolarization and activation of intracellular cascades through Ca^{2+} influx [122]. In this regard, the downregulation of α7 nAChRs might preclude the anti-inflammatory reflex. Opposite to this mechanism, the upregulation of α4β2 nAChRs elicited by nicotine is a very well characterized process [123] that is more prevalent in smokers [124].

An important evidence of immune modulation of centrally acting α7 nAChRs is that the activation of these receptors regulates the production of cytokines [125, 126], and, in turn, cytokine levels affect not only neuronal survival [127], but also neuronal function. For example, the pro-inflammatory cytokines TNF-α and interleukin (IL)-1 and IL-6, whose levels are determined by the extent of local and systemic immune activation, directly modulate the amplitude of excitatory and inhibitory synaptic currents, respectively [61, 128, 129]. This observations supply an explanation why the activation of the anti-inflammatory reflex exerts a protective effect on the IL-6-mediated decrease of synaptic inhibition induced by LPS [130], which has been proposed to be an important factor in the onset of hyperexcitable neuropsychiatric conditions including epilepsy, anxiety disorders, depression [60], and schizophrenic psychosis [131].

The clinical relevance of these findings is highlighted by the existence of a line of work which is testing the hypothesis that an enhancement of the anti-inflammatory reflex may represent a viable strategy to reduce damage from different types of insult originated from inflammation or immune activation.

Given the relevance of the interactions between inflammation and neuropsychiatric disease discussed above [57, 132-140], it is of obvious importance for neuroscience research in the next decade to investigate the existence of an analogue of the anti-inflammatory reflex in the CNS and to look for pharmacological or neurological tools to activate or enhance the CNS anti-inflammatory reflex in the search for more effective treatments for neuropsychiatric conditions.

Given that pro-inflammatory cytokines like TNFα and IL-6, decreases the ratio between synaptic inhibition and excitation [141, 61], and because of their anti-inflammatory role, α7 nAChRs have been proposed important potential targets for antidepressants [62]. In agreement with this line of work, diverse vagal nerve stimulations, pharmacologically using nicotinic agonists, or electrically, the so-called vagal nerve stimulation therapy, decrease inflammation. This mechanism might also explain, or at least be a factor, in the effectiveness of the vagal nerve stimulation (VNS) therapy in the treatment of clinical depression, recently approved by the FDA.

A novel mechanism of action for α7 nAChRs

While the high-Ca^{2+} permeability of α7 nAChRs, associated with the activation of numerous intracellular cascades is commonly interpreted as the responsible factor for most of the α7 nAChR-linked biological effects, recent studies have proposed a novel anti-inflammatory mechanism of action mediated by these receptors in a partly Ca^{2+}-independent manner [142]. This is based on the discovery of α7 nAChRs on the outer mitochondrial membrane [143-145]. According to this hypothesis, ACh or other nAChR agonists -including nicotine- accumulates into the cytoplasm of macrophages upon adenosine tri-phosphate (ATP) stimulation and binds to α7 nAChRs on the mitochondrial outer membrane, inhibiting the NACHT, LRR, NYP domains-containing protein 3 (NLRP3) inflammasome, a mitochondrial molecular complex mediating innate immunity response [142]. The inhibition of NLRP3 inflammasome reduces the mitochondrial release of cytotoxic molecules like hydrogen oxide anions and mitochondrial DNA into the macrophage cytoplasm, which, in turn, reduce or stop ATP-induced activation of the pro-apoptotic caspase 1 and the release of pro-inflammatory products, including interleukin-1β (IL-1β) and extracellular high mobility group box 1 (HMGB1) (Fig. 2). The eventual effect of nicotinic agonists is the blockade of an inflammation-triggered cell death sequence similar to apoptosis, denominated pyroptosis [146, 147].

While -to our knowledge- this process has only been demonstrated in peripheral macrophages (i.e., marrow bone dendritic cells) [142], it is tempting to speculate that a similar mechanism may take place in microglia, which are the resident macrophages of the CNS, or in macrophages infiltrating the brain following pathological or iatrogenic permeabilization of the BBB. Such phenomenon might be and additional or alternative factor contributing to the enhanced rate of smoking observed among neuropsychiatric sufferers of inflammation-related brain disease, and perhaps contributing to neurodegeneration associated with the loss of cholinergic forebrain neurons observed in the early stages of various neurodegenerative illnesses [148, 149], particularly AD [150, 151] and Parkinson's disease (PD) [152, 153]. Incidentally, this interpretation also supplies a reasonable explanation for the limited effectiveness offered by the symptomatic treatment of AD with AChE inhibitors, which does not increase the availability of ACh to non-neuronal cells.

α7 AChRs, brain inflammation, and neurodegeneration

The link between neurodegenerative disease and brain inflammation deserves a special emphasis in our discussion. In fact, while the hypothesis of a connection between neuropsychiatric acute conditions and inflammation is gaining momentum with increasing wealth of evidence, a connection between inflammation, pathological plasticity, and neurodegeneration has been confirmed by numerous observations, starting with the early findings that brain inflammation is elevated in AD and PD patients, as well as in multiple sclerosis (MS) patients [154].

The activation of α7 nAChRs has been reported to be antiapoptotic in nature [155-159]. A specific role of α7 nAChRs in neuroprotection has been addressed [160], associated with the high permeability to Ca^{2+} of these receptors. Examination of postmortem brains from both AD and PD patients shows a decrease of α7 nAChR content in the temporal cortex [161]. It is not clear yet whether chronic brain inflammation is secondary to the presence of these conditions, or is rather primary in its etiology. In this regard, the absence of a neuroprotective effect of nicotine administered after (but not before) stereological injection of the dopaminergic neurotoxin 6-hydroxy dopamine [162], or after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) damage [163], corroborates the irreversibility of the toxin damage, and indirectly supports a primary role of oxidative stress in neurodegeneration, reinforcing a potential use of α7 nAChR agonists in the treatment of these neurodegenerative diseases. A study with PC12 cells using specific α7 agonists suggests that the neuroprotective effect of nicotine by activation of α7 nAChRs in AD is potentially due to the expression of the anti-apoptotic protein Bcl-2 through an intracellular cascade involving the inflammatory effectors JAK-2 and NF-kB [164].

While a neuroprotective effect of nicotine in AD or other neurodegenerative disorders has not been shown -to our knowledge- in general terms, a study utilizing co-cultures of microglia and dopaminergic mesencephalic neurons has shown that nicotine decreases inflammation-related neuronal death caused by LPS [165]. A decrease in neuroinflammation associated with an increase in the expression of α7 nAChRs might explain the neuroprotective effect of neuregulins [121], whose activity also modulates other neuronal functions. α7

nAChR agonists produce a neuroprotective action by decreasing the elevation of proinflammatory cytokines caused by icv LPS administration through a PI$_3$K-dependent cascade [166]. Importantly, the α7 nAChR activation also decreases the phosphorylation of the tau protein, which is a major intracellular marker for AD [167]. The administration of selective α7 nAChR agonists exerts a neuroprotective action also in an animal model of intracerebral hemorrhage by autologous blood infusion, with a mechanism dependent on the glycogen synthase kinase 3β, corroborating a link between neuroinflammation and neuroprotection in mood disorders [168].

Previous animal studies comparing pain-related behaviors between wild-type and α7 mutant mice, including knockout α7-/- as well as α7-L250T hypersensitive mice, using a variety of acute, chronic inflammatory, and neuropathic models, support a functional role of α7 nAChRs in chronic neuropathic and inflammatory pain [169]. Interestingly, the use of PAMs with selectivity for α7 nAChRs has been successfully tested in several rodent models of inflammatory and chronic neuropathic pain [170]. In particular, PNU-120596, a type II PAM (i.e., potentiates agonist-induced α7 nAChR activity and decreases receptor desensitization; reviewed in [77]), shows significant anti-edematous and anti-allodynic effects in inflammatory and chronic constriction injury pain models [170], and alleviates inflammatory hyperalgesia and cytokine release [171]. In addition, using an animal model of focal cerebral ischemia, PNU-120596 reduced neuronal damage and improved behavioral function [172, 173]. Finally, PAM-2, a novel type II PAM [174], with antidepressant activity [175], produces antinociceptive and anti-inflammatory effects [176].

Conclusions

Mounting evidence supports the view that α7 nAChRs are involved in the process of neuroinflammation and peripheral inflammation. In this regard, α7 nAChR agonists can target immune cells either or both in the periphery or within the CNS, whereby decreasing the detrimental effects of inflammation elicited by an overactivity of the cytokine network. This effect maybe explained along the same terms as the physiological effects of endogenous ACh (i.e., inside the CNS as well as a consequence of direct and indirect actions of the autonomic system), but also in supplying an alternative route of action of exogenous nicotinic agonists such as nicotine itself. Two main avenues of α7 nAChR stimulation in the immune system are currently available: electric stimulation, the so-called vagal nerve stimulation therapy, and pharmacological activation, through the use of α7 nAChR agonists. Among the latter, the use of selective agonists and PAMs appears recommendable as it minimizes the side effects otherwise associated with the use of other less selective compounds. For all these reasons, treatments using α7 nAChR agonists and PAMs have potential for the treatment of neurodegenerative conditions as well as in neuropsychiatric stress-related diseases that have not yet fully explored.

Conflicting interests

The authors have declared that no conflict of interests exists.

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Author contributions

M.A. wrote the initial version of the MS, F.G-O and H.A. improved the text and performed changes. All authors contributed intellectually and practically to the work.

Abbreviations

nAChR: Nicotinic acetylcholine receptor; mAChR: Muscarinic acetylcholine receptor; CNS: central nervous system; GABA: γ-aminobutyric acid; NMDA: N-methyl-D-aspartate; BBB: blood brain barrier; icv: intracerebroventricular; IP$_3$K: inositol triphosphate kinase; MIA: maternal inflammatory activation; LPS: lipopolysaccharide; SSRI: serotonin selective reuptake inhibitor; NSRI: norepinephrine selective reuptake inhibitor; ASD: autism spectrum disorder; PV+: parvalbumin positive; VNS: vagal nerve stimulation; DNA: deoxyribonucleic acid.

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