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Activation of the Macrophage α7 Nicotinic Acetylcholine Receptor and Control of Inflammation

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Abstract

Inflammatory responses to stimuli are essential body defenses against foreign threats. However, uncontrolled inflammation may result in serious health problems, which can be life-threatening. The α7 nicotinic acetylcholine receptor, a ligand-gated ion channel expressed in the nervous and immune systems, has an essential role in the control of inflammation. Activation of the macrophage α7 receptor by acetylcholine, nicotine, or other agonists, selectively inhibits production of pro-inflammatory cytokines while leaving anti-inflammatory cytokines undisturbed. The neural control of this regulation pathway was discovered recently and it was named the cholinergic anti-inflammatory pathway (CAP). When afferent vagus nerve terminals are activated by cytokines or other pro-inflammatory stimuli, the message travels through the afferent vagus nerve, resulting in action potentials traveling down efferent vagus nerve fibers in a process that eventually leads to macrophage α7 activation by acetylcholine and inhibition of pro-inflammatory cytokines production. The mechanism by which activation of α7 in macrophages regulates pro-inflammatory responses is subject of intense research, and important insights have thus been made. The results suggest that activation of the macrophage α7 controls inflammation by inhibiting NF-κB nuclear translocation, and activating the JAK2/STAT3 pathway among other suggested pathways. While the α7 is well characterized as a ligand-gated ion channel in neurons, whole-cell patch clamp experiments suggest that α7's ion channel activity, defined as the translocation of ions across the membrane in response to ligands, is absent in leukocytes, and therefore, ion channel activity is generally assumed not to be required for the operation of the CAP. In this perspective, we briefly review macrophage α7 activation as it relates to the control of inflammation, and broaden the current view by providing single-channel currents as evidence that the α7 expressed in macrophages retains its ion translocation activity despite the absence of whole-cell currents. Whether this ion-translocating activity is relevant for the proper operation of the CAP or other important physiological processes remains obscure.

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Keywords

Alpha7 nAChR; Cholinergic anti-inflammatory pathway; Inflammation; Nicotinic acetylcholine receptor; Monocyte-derived macrophages (MDMs)

Introduction

Inflammation is the physiological response to invading pathogens and tissue damage. The inflammatory response leads to engagement of the immune system by the release of pro-inflammatory cytokines to the bloodstream. Well-controlled inflammation helps clear invading pathogens and promotes tissue repair thus reaching homeostasis. However, uncontrolled inflammation may generate more problems than it solves as it may cause tissue injury, organ dysfunction and death. Known mediators of inflammation resolution include anti-inflammatory cytokines, which inhibit the effect of the pro-inflammatory cytokines; and lipoxins and resolvins, among others, that promote tissue healing. These mediators of inflammation resolution reach their target by diffusion and thus their intended effect does not take place immediately. One of the most interesting pathways for the regulation of inflammation is the cholinergic anti-inflammatory pathway (CAP) as it involves the nervous and immune systems. The anti-inflammatory effect of this pathway requires the release of neurotransmitters norepinephrine and acetylcholine, and it has the advantage that the anti-inflammatory signals can reach target organs to respond to threat immediately. The interplay of the nervous system and immune cells to fine-tune inflammatory responses is a relatively recent discovery and it is known as the inflammatory reflex. The efferent arm of the inflammatory reflex is called the cholinergic anti-inflammatory pathway (CAP).

Cholinergic Anti-Inflammatory Pathway

The CAP has been the subject of intense research since its discovery more than a decade ago. The CAP comprises an interaction between the nervous and immune systems that regulate inflammatory responses. It fine-tunes the response to inflammatory stimuli to reach homeostasis and potentially avoid organ damage and death. The pathway is initiated through afferent vagus nerve stimulation by lipopolysaccharide (LPS), a bacterial endotoxin, or pro-inflammatory cytokines. The signal travels to the brain where it is processed in a muscarinic acetylcholine receptor-dependent mechanism. The integrated anti-inflammatory signal is conveyed through vagus nerve efferent fibers reaching the celiac-superior mesenteric plexus (CSMP). The vagus nerve efferent fiber originating in the dorsal motor nucleus is connected to the splenic nerve in the CSMP, and conveys the anti-inflammatory signal to the spleen, where the splenic nerve terminals release norepinephrine (NE) activating ß2 adrenergic receptors in specialized T-lymphocytes that express choline acetyltransferase ($ChAT^+$ T cells) and synthesize ACh (Rosas-Ballina et al. 2011). The NE-activated $ChAT^+$ T cells travel to macrophages in the spleen and release ACh. Activation of the α7 nicotinic receptors in macrophages by the ACh released by the $ChAT^+$ T cells causes a downregulation in the production and release of pro-inflammatory cytokines (Fig. 1), whereas anti-inflammatory cytokines levels are left unchanged (Borovikova et al. 2000).

Nicotinic Acetylcholine Receptors

The nicotinic acetylcholine receptor (nAChR) is a member of the superfamily of Cys-loop ligand-gated ion channels which includes the glycine receptors, the γ-aminobutyric acid type A receptors ($GABA_A$) and the serotonin 5-HT_3 receptors (Cockcroft et al. 1990; Corringer et al. 2000; Karlin 2002; Le Novère et al. 2002). It is responsible for the relay of action potentials across cholinergic synapses through binding of the neurotransmitter acetylcholine (ACh) (Karlin 2002; Le Novère et al. 2002). Upon ACh binding, the nAChR undergoes conformational changes resulting in the opening of its cation-selective central pore. The nAChR is profusely expressed in the electric organs found in electric rays such as those from the genus *Torpedo* where they mediate the electric discharge used by rays to stun or kill prey (Cartaud et al. 2000).

The nAChR is an integral membrane protein composed of five subunits arranged as a five-member ring, the center of which is the receptor's cation-selective pore (Ochoa et al. 1989; Cockcroft et al. 1990; Galzi et al. 1991; Changeux et al. 1992; Karlin and Akabas 1995; Arias 1998; Changeux and Edelstein 1998; Corringer et al. 2000; Karlin 2002; Le Novère et al. 2002). Each subunit contains four hydrophobic transmembrane domains designated M1 through M4, a long extracellular N-terminal, a long cytoplasmic loop between M3 and M4 and a short extracellular C-terminus (DiPaola et al. 1989; Devillers-Thiéry et al. 1993). The nAChR is present in presynaptic neurons, postsynaptic neurons, muscle cells and electrocytes, the cells from the electric ray organ of electric rays (Galzi et al. 1991; Changeux and Edelstein 1998). In addition, nicotinic receptors have been identified in other cells such as those from the immune system, among others. Neuronal nAChRs may comprise only α subunits (homooligomeric) or a combination of α and β subunits (heterooligomeric) (Galzi et al. 1991; Cooper et al. 1991; Zhang et al. 1994). Adding to this level of complexity, neuronal nAChRs employ a variety of α and β subunits (Paterson and Nordberg 2000; Itier and Bertrand 2001). Thus far, ten α subunits designated α1 through α10 and four β subunits designated β1 through β4 have been described (Le Novère et al. 1999; Kalamida et al. 2007).

α7 Nicotinic Acetylcholine Receptor

The α7 receptor is an homooligomeric nAChR that is abundantly expressed in the central nervous system (CNS). The α7 is characterized by its fast desensitization and high calcium permeability. It is involved in learning and memory, and implicated in neurological disorders such as Parkinson's disease, Alzheimer's disease, and schizophrenia that may result from decreased expression or functionality of this receptor. In addition, the α7 is also expressed in non-neuronal cells. For instance, activation of the α7 in lung and pancreatic cancers has been demonstrated to promote angiogenesis, cell-cycle progression and metastasis (Schaal and Chellappan 2014). Along this line, α7 antagonists have been suggested as potential treatment for lung cancer (Brown et al. 2012). The α7 is also expressed in cells from the immune system such as lymphocytes, monocytes and macrophages. Indeed, ACh-induced activation of α7 in macrophages stimulate the so-called cholinergic anti-inflammatory pathway (Wang et al. 2003).

Essential Role of the α7 Nicotinic Receptor in the CAP

The role of the α7 nicotinic receptor is essential to the CAP-mediated regulation of pro-inflammatory responses (Wang et al. 2003). The CAP has been studied in human monocyte-derived macrophages (MDMs) exposed to LPS, wherein the concentration of pro-inflammatory cytokines TNF-α, IL-6, IL-1β, and anti-inflammatory cytokine IL-10 was determined in MDMs exposed to ACh and pyridostigmine, an acetylcholinesterase inhibitor, before being challenged by the LPS inflammatory stimulus (Borovikova et al. 2000; Wang et al. 2003). These experiments demonstrated that, in MDMs pre-treated with pyridostigmine and ACh, LPS exposure failed to increase the concentration of released TNF-α, IL-6, and IL-1β whereas the concentration of anti-inflammatory cytokine IL-10 remained unchanged. To ascertain the role of the α7 nicotinic receptor in the anti-inflammatory effect of ACh on macrophages, experiments in which the α7 receptor was either silenced or knocked-out were performed. These experimental approaches revealed that the α7 nicotinic receptor is essential in the CAP-mediated regulation of inflammatory responses as the pathway ceases to work if macrophages are exposed to antisense oligonucleotides specific for the α7, or if the gene that codes for the α7 receptor (*CHRNA7*) is knocked-out (Wang et al. 2003).

Cholinergic Anti-Inflammatory Pathway Signaling

The underlying mechanism of the CAP has been the subject of intense research by several groups (Sundman and Olofsson 2014). Studies suggest potential mechanisms whereby activation of the α7 expressed in macrophages suppresses the production and release of pro-inflammatory cytokines. For the most part, the proposed mechanisms implicate NF-κB and JAK2-STAT3 signaling pathways (see Fig. 1); however, more recent insights into the CAP underlying mechanism reveal roles for other molecules and signaling cascades.

NF-κB Nuclear Translocation—Experiments demonstrated that activation of the α7 nicotinic receptor in macrophages suppresses inflammation by inhibition of nuclear translocation of transcription factor NF-κB (Wang et al. 2004). The nuclear factor KB (NF-κB) transcription factor participates in the regulation of inflammatory processes by activating the expression of pro-inflammatory cytokines. It responds quickly to pro-inflammatory stimuli because it needs not be produced upon stimuli as it is maintained in the cytoplasm in an inactivated form. The NF-κB subunits p50 and p65 are maintained inactivated by binding to I-κB. When toll-like receptor (TLR4) is activated by ligands such as pro-inflammatory cytokines and LPS, I-κB is phosphorylated by IKK and signaled for degradation. With I-κB phosphorylated, NF-κB subunits p50 and p65 are free to translocate to the cell nucleus where they activate the transcription of pro-inflammatory cytokines, which amplify the inflammatory response mounted by the body in response to a pro-inflammatory stimulus (Fig. 1, *dark green arrows*). Therefore, an anti-inflammatory pathway meant to fine-tune the inflammatory response could do so by inhibiting the nuclear translocation of NF-κB. Indeed, research shows that activation of the α7 and hence the cholinergic anti-inflammatory pathway, prevents NF-κB nuclear translocation in macrophages thus inhibiting the secretion of high mobility group box 1 (HMGB1), an important pro-inflammatory cytokine that serves as late mediator of sepsis (Wang et al. 2004). Furthermore, administration of nicotine to experimental models of sepsis reduced

serum HMGB1 levels and improved survival rates (Wang et al. 2004). The exact mechanism whereby activation of α7 in macrophages results in the blockade of NF-κB nuclear translocation is still not clear. However, nicotine-induced inhibition of Iκ-B phosphorylation by IKK and suppression of NF-κB transcriptional activity have been demonstrated (Fig. 1, *red lines*) (Yoshikawa et al. 2006). In addition, activation of the Jak2/STAT3 signaling cascade, discussed below, may converge with the NF-κB pathway to inhibit NF-κB (Peña et al. 2010). Indeed, it has been suggested that the α7-mediated inhibition of NF-κB may result from its interaction with unphosphorylated STAT3 (uSTAT3) (Yang et al. 2007; Peña et al. 2010).

Jak2/STAT3 Signaling Cascade—Activation of the α7 in macrophages may fine-tune pro-inflammatory responses by activating the Jak2/STAT3 signaling cascade. Two alternative models have been proposed to illustrate the role of the Jak2/STAT3 pathway in the CAP (de Jonge et al. 2005; Peña et al. 2010). The first model states that binding of cholinergic agonists to the α7 results in the recruitment of Jak2 to the α7. Jak2 autophosphorylates itself, STAT3 is then recruited and phosphorylated by Jak2, and pSTAT3 form dimers that translocate to the nucleus (Fig. 1, *yellow arrows*). The mechanism by which pSTAT3 dimers impede the production and release of pro-inflammatory cytokines is not completely understood although STAT3 acts as negative regulator of the pro-inflammatory response (de Jonge et al. 2005). The authors of this study suspected that SOCS3 could be implicated in the mechanism because the expression of SOCS3 is activated by STAT3 and because SOCS3 inhibits the Jak2 phosphorylation of STAT3. Nevertheless, experiments demonstrated that blockade of the SOCS3 expression did not preclude the operation of the CAP. The authors thus concluded that the mechanism of the CAP is related to enhanced STAT3 instead of SOCS3 activation. A recent study shed light on the mechanism by which activation of STAT3 by nicotine may result in the observed anti-inflammatory effects (Joe et al. 2011). In experiments performed using U937 cells—a monocytic cell line–, nicotine-activated STAT3 was demonstrated to induce the production of tristetraprolin (TTP), an AU-rich element (ARE)-binding protein (Fig. 1, *blue arrows*). The induced production of TTP was suggested to generate the cholinergic anti-inflammatory effect by destabilizing pro-inflammatory transcripts containing AREs in the 3′-untranslated region (3′-UTR) (Joe et al. 2011).

Peña et al. introduced a novel perspective on the mechanism for the cholinergic regulation of inflammation that is related to the JAK2/STAT3 signaling cascade. In their model, unphosphorylated STAT3 (uSTAT3) and not pSTAT3 is responsible for the anti-inflammatory response (Peña et al. 2010). Their results suggest that the cholinergic anti-inflammatory response results from inhibition of STAT3 tyrosine phosphorylation (Peña et al. 2010) (Fig. 1, *light green arrows*). It is noteworthy that these experiments were performed using choline as agonist, whereas the previous study (de Jonge et al. 2005) implicating pSTAT3 as having a role in the underlying mechanism of the CAP were performed using nicotine as agonist. According to the authors, uSTAT3 interferes with the LPS-induced pro-inflammatory response via binding of NF-κB thus displacing I-KB and forming a complex (uSTAT3-NF-κB) that had been previously described in the literature

(Yang et al. 2007). The authors speculated that the uSTAT3-NF-κB complex could prevent NF-κB activation thus bringing about the anti-inflammatory effect of cholinergic agonists.

Heme Oxygenase-1 Induction—An alternative mechanism for the cholinergic regulation of pro-inflammatory responses is mediated by the upregulation of heme oxygenase-1 (HO-1) (Fig. 1, *purple arrow*). In experiments performed using RAW264.7 cells, nicotine dose-dependently increased HO-1 expression in an α7-dependent manner as the nicotine-induced upregulation of HO-1 was obliterated when α7 was either antagonized using mecamylamine, or silenced by RNA interference. According to this study, the upregulation of HO-1 produced by activation of the α7 nicotinic receptor requires Ca^{2+} influx because chelators EGTA and BABTA dose-dependently reduced the nicotine-induced HO-1 upregulation. The signaling cascade that is initiated by activation of the α7 receptor and the concomitant Ca^{2+} influx and that ends in an anti-inflammatory effect comprise activation of classical PKC by a Ca^{2+}-dependent mechanism, an increase in reactive oxygen species (ROS) production in a process involving NADPH oxidase, and the activation of the phosphoinositol-3-kinase (PI3K)/Akt/Nrf-2 pathway, which induces the expression of HO-1 in macrophages. The nicotine-induced upregulation of HO-1 was suggested to be an important step in the anti-inflammatory effect of nicotine in macrophages (Tsoyi et al. 2011).

Alternative PI3K-Related Mechanisms—Recent studies shed additional light on alternative mechanisms of the cholinergic control of inflammation that involve PI3K signaling. In experiments performed in RAW264.7 cells stimulated with LPS, activation of the α7 with nicotine was demonstrated to suppress TLR4 expression through PI3K/Akt activation. These results were suggested to imply that the protective effect of nicotine could also be associated with inhibition of LPS-induced TLR4 overexpression (Kim et al. 2014). In addition, an alternative mechanism for the CAP was put forward involving interleukin-1 receptor-associated kinase M (IRAK-M), which is a negative regulator of innate TLR-mediated immune responses (Maldifassi et al. 2014). According to the proposed mechanism, nicotine induces the upregulation of IRAK-M in macrophages through a single (JAK2/PI3K/STAT3) or two convergent cascades (JAK2/STAT3 and PI3K/STAT3). The anti-inflammatory effect of α7 agonists is due, as proposed by the authors, to the upregulation of IRAK-M, which has an anti-inflammatory effect, and to the observed nicotine-induced reprogramming of macrophages to become refractory and hyporesponsive to TLR stimulation (Maldifassi et al. 2014).

The α7 nAChR Ion Translocation Activity

The α7 belongs to the family of ligand-gated ion channels and its ion translocation activity has been characterized extensively by electrophysiological methods in various expression systems including *Xenopus* oocytes, mammalian cells, and neurons (Papke 2014). Binding of agonists such as acetylcholine and nicotine produces conformational changes in the receptor that open its central pore allowing the flow of cations from one side of the cell membrane to the other. The flow of positive charges along the electrochemical gradient changes the membrane potential. The α7 is characterized by very fast desensitization and by its high calcium permeability. Therefore, activation of this nicotinic receptor can initiate

calcium-dependent signaling cascades (Shen and Yakel 2009). While the ion translocation activity of the α7 has been extensively studied by various electrophysiological techniques, whole-cell patch clamp studies performed in peripheral blood mononuclear cells (Villiger et al. 2002; Skok 2009) have failed to demonstrate α7 macroscopic currents in these cells. While previous studies have reported the essential role of the α7 in the regulation of inflammation (Wang et al. 2003) none have shown, to the best of our knowledge, evidence of α7 ion translocation activity in macrophages.

In this perspective we provide single-channel currents suggesting that the α7 expressed in human MDMs retains its ion channel activity—defined as the translocation of ions across the membrane in response to agonists—despite the absence of whole-cell currents.

Materials and Methods

Monocyte-Derived Macrophages Culture

All donors enrolled in the study signed the approved informed consent. Whole blood from healthy subjects was processed as described elsewhere (Delgado-Vélez et al. 2008). Peripheral blood mononuclear cells were counted by hemocytometer, adjusted to 1×10^6 cells/ml and seeded in 12 mm round No. 1 coverslips (Warner Instruments). Monocytes were separated from lymphocytes by adherence and differentiated for 7–8 days in RPMI-1640 supplemented with 20 % inactivated FBS (Sigma-Aldrich), 10 % inactivated human serum (Sigma-Aldrich), 2 mg/ml MCSF (Invitrogen), and 1 % PenStrep (Sigma-Aldrich). All cultures were maintained at 37 °C with 5 % CO_2.

Cell-Attached Patch Clamp

MDMs were placed in a recording chamber containing bath solution (140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 5 mM HEPES, pH 7.4) at 20 to 22 °C. The patch pipettes were pulled from thick-walled borosilicate glass (Sutter Instruments). Single-channel currents were recorded in the cell-attached patch clamp configuration using an Axopatch 200B amplifier (Molecular Devices, LLC), filtered at 5 kHz and digitalized at 50 kHz using the pClamp 10.4 Clampex software (Molecular Devices, LLC). The concentration of ACh, choline and PNU-120596 were 30 μM, 15 μM, and 10 μM, respectively.

Results and Discussion

It has been previously reported that ACh does not elicit macroscopic currents in immune cells that express the α7 nicotinic receptor (Villiger et al. 2002; Skok 2009), an observation that we too have made (data not shown). Nonetheless, our results demonstrate that the macrophage α7 retains its ion translocation functionality as judged by single-channel recordings performed on human MDMs. Figure 2 shows patch clamp experiments in the cell-attached configuration performed in MDMs which revealed single-channel currents that are consistent with published α7 currents (daCosta et al. 2011). The α7 is known for its fast desensitization, a characteristic that may hinder single-channel recordings. If the concentration of the agonist is too high, the receptor becomes desensitized, and if the concentration is too low, the frequency of single-channel openings may not be appreciable.

After trying a few ACh concentrations in the 0.03–100 μM range, the 30 μM seemed to produce a good compromise. As displayed in Fig. 2a, these recordings revealed single-channel events of short durations characteristic of the α7 receptor.

PNU-120596 is a type II positive allosteric modulator that extends the openings of the α7 in addition to reverting the desensitized state (daCosta et al. 2011). Developed by Pfizer, PNU-120596 selectively modulates the activity of the α7 leaving the functionality of other nicotinic receptors unmodified (Hurst et al. 2005). PNU-120596 (10 μM) increased the frequency of ACh-induced openings in addition to a substantial prolongation of the open state suggesting that the single-channels correspond to the α7 (Fig. 2b and c).

As shown on Fig. 2d, choline can activate the α7 in human macrophages at a concentration of 15 μM. Addition of the α7 receptor antagonist α-bungarotoxin obliterated the choline-elicited currents (See Online Resource 1). Furthermore, our preliminary data suggest that PNU-120596 extends the choline-induced α7 single-channel openings in macrophages (See Online Resource 2). Choline is a full agonist of the α7 and its concentration in the blood expands from 8.6 to 141.4 μM (Alkondon et al. 1997; Lueders et al. 2007). Therefore, the result summarized on Fig. 2d implies that physiologically relevant concentrations of choline can activate the macrophage's α7.

While macroscopic currents are not observed in macrophages, it is possible that these may not be necessary to exert physiological responses. For instance, it is clear from the literature that activation of the macrophage α7 is essential to reduce the secretion of pro-inflammatory cytokines through the CAP even when α7 macroscopic currents have not been detected in these cells (Wang et al. 2003). However, the following question persists: Is ion channel activity—albeit not macroscopic currents—necessary for the role of the α7 in the control of inflammation? Our results described on Fig. 2d suggest that the ion channel activity detected at the single-channel level is not sufficient for CAP operation because choline elicits α7 single-channel currents at a concentration of 15 μM; however, choline does not result in a reduction in the release of TNF-α in human MDMs at concentrations below 1 mM (Parrish et al. 2008). Nevertheless, while ion channel currents at the single-channel level may not be sufficient to initiate the CAP, their potential role in regulating the CAP or other important physiological processess remain to be elucidated.

Conclusion

Pathogens trigger immunological responses involving inflammatory processes orchestrated by pro-inflammatory and anti-inflammatory mediators. This formidable mechanism helps eliminate harmful agents preventing organ and systemic damage. However, exaggerated and uncontrolled sustained inflammatory responses may lead to series health problems. The α7 nicotinic receptor expressed in macrophages plays an essential role in the regulation of inflammatory processes. The ionotropic activity of the α7 nicotinic receptor has been ruled out as relevant to the operation of the CAP mainly on the grounds that macroscopic currents have not been recorded in nonexcitable immune cells (Villiger et al. 2002; Skok 2009; Papke 2014). Thus, metabotropic roles are ussually ascribed to α7-mediated regulation of inflammatory responses through the CAP. The underlying mechanisms involves NF-κB, and

JAK2/STAT3 signaling cascades (de Jonge et al. 2005). In addition, mechanims involving TTP, HO-1, PI3K, TLR4 and IRAK-M have been implicated in the CAP (Tsoyi et al. 2011; Joe et al. 2011; Maldifassi et al. 2014; Kim et al. 2014).

While α7 macroscopic currents are not observed in nonexcitable cells suchs as immune cells, these may not be essential for the potential ionotropic functions of the α7 in these cells. For instance, translocated calcium through the α7 ion channel can produce calcium-induced calcium release processes that could initiate calcium-dependent pathways (Gilbert et al. 2009; Shen and Yakel 2009). In our laboratory we have replicated the observation that MDMs do not produce ACh-activated macroscopic currents detectable in whole-cell patch clamp experiments (data not shown). Nevertheless, we did observe the characteristic α7 single-channel currents responsive to PNU-120596 when performing cell-attached single-channel patch clamp experiments (Fig. 2).

The difficulties associated with recording whole-cell α7 currents in macrophages hints that this receptor may be subject to stringent regulation. The underlying mechanism behind the hypothesized regulation of the α7 in MDMs may include intracellular regulation by threonine/serine (Russo and Taly 2012) or tyrosine (Charpantier et al. 2005) phosporylation, or by cytoplasmic or membrane-anchored proteins (Paulo et al. 2009). In addition, α7 nicotinic acetylcholine receptor activity can be regulated by cytoplamic calcium concentrations (Shen and Yakel 2009). A compelling explanation to account for the lack of α7 macroscopic currents in MDMs is provided by the demonstration that macrophages abundantly express a partial duplication of the gene that codes for the α7 (*CHRNA7*), namely the *CHRFAM7A* (Benfante et al. 2011; de Lucas-Cerrillo et al. 2011). This gene encodes the dupα7 protein which has been demonstrated to serve as a negative regulator of the α7 when co-expressed in *Xenopus* oocytes, allthough this view has been recently challenged using Neuro2a cells as expression system (Wang et al. 2014). Nevertheless, co-expression of α7 and dupα7 in *Xenopus* oocytes in a 1:5 ratio results in a truly remarkable reduction in α7 macroscopic currents. This reduction would be expected to be even greater in macrophages as the α7 mRNA levels are only 7 % of the dupα7 mRNA levels (de Lucas-Cerrillo et al. 2011).

In conclusion, we provide electrophysiological evidence that the α7 expressed in macrophages functions as an ion-conducting channel. Whether the observed ion translocation activity of the α7 expressed in MDMs has a role in the underlying mechanism of the CAP remains obscured and would required further research to ascertain. Nevertheless, knowledge that the macrophage α7 retains its ion translocation functionality implies that the ample research focused on modulating the ion channel activity of the α7 receptor could be pertinent to the macrophage's α7.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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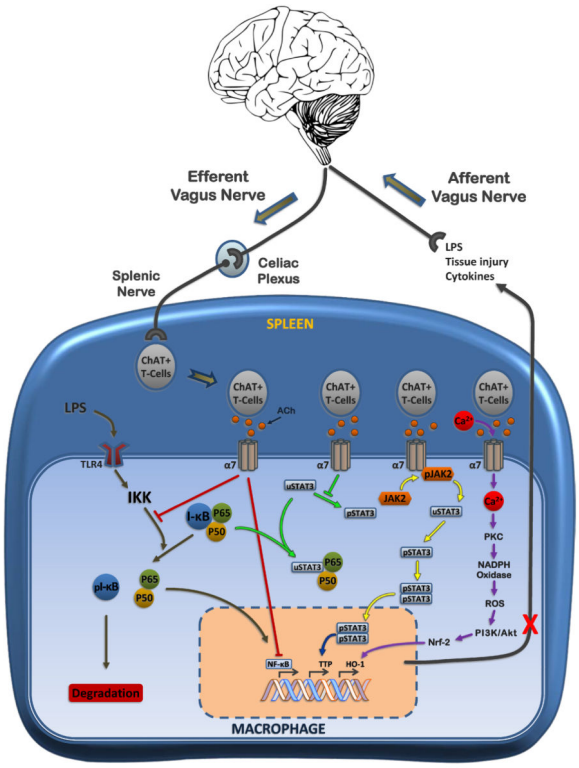

Fig. 1.

The cholinergic anti-inflammatory reflex. Invasion of pathogens and tissue damage produce inflammatory responses that are regulated in part by the cholinergic anti-inflammatory reflex. Endotoxins or pro-inflammatory cytokines signal through vagus nerve afferent fibers to the brain wherein the message is processed, resulting in an anti-inflammatory signal transmitted through vagus nerve efferent fibers to the celiac plexus. The splenic nerve then activates T-cells that express choline acetyltransferase (ChAT$^+$ T-cells) in the spleen, which travel to macrophages and secrete ACh activating their α7 receptors. The efferent arm of the anti-inflammatory reflex is known as the cholinergic anti-inflammatory pathway (CAP). Activation of the α7 inhibits NF-κB by interfering with I-κB phosphorylation and NF-κB transcriptional activity (*red lines*). Activation of the α7 has been proposed to inhibit STAT3 phosphorylation, leaving uSTAT3 available to bind NF-κB subunits p50 and p65, and avoiding their nuclear translocation (*light green arrows*). On the other hand, it has been reported that the anti-inflammatory effect that results from activation of α7 is due to activation of the Jak2/STAT3 signaling pathway (*yellow arrows*). In a separate work, activated STAT3 was shown to induce the production of tristetraprolin (TTP), which is suggested as mediator of the anti-inflammatory effect (*blue arrow*). An additional proposed mechanism for the cholinergic regulation of pro-inflammatory responses comprise the upregulation of heme oxygenase-1 (HO-1) mediated by the activation of the phosphoinositol-3-kinase (PI3K)/Akt/Nrf-2 pathway (*purple arrows*)

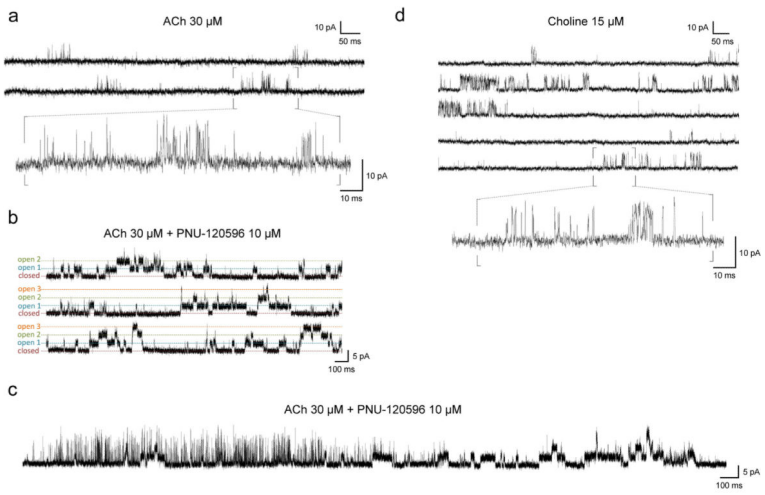

Fig. 2.

Patch clamp characterization of the α7 in MDMs. **a** Patch clamp experiments performed in the cell-attached configuration, using ACh (30 μM) as agonist demonstrate that the α7 expressed in MDMs function as ion channels. **b** and **c** Exposure to selective α7 positive allosteric modulator PNU-120596 (10 μM) results in prolonged openings confirming that currents are indeed from α7. **c**. This trace shows how PNU-120596 first increases the frequency of openings consistent with previous observations by daCosta et al. 2011. All experiments were performed at a holding potential of −100 mV. **d**. Patch clamp experiments performed in the cell-attached configuration, and using choline (15 μM) as agonist further demonstrates that the α7 expressed in MDMs behaves as a ligand-gated ion channel