α7 Nicotinic Acetylcholine Receptor and Neuroinflammation

Subjects: Neurosciences

Contributor: Tehila Mizrachi

α7 is a Nicotinic acetylcholine receptor (nAChRs) that is composed of five identical α7 subunites.Those receptors are widely expressed in or on various cell types and have diverse functions. In immune cells nAChRs regulate proliferation, differentiation and cytokine release. Specifically, activation of the α7 nAChR reduces inflammation as part of the cholinergic anti-inflammatory pathway.

Keywords: cholinergic anti-inflammatory pathway ; neuroinflammation ; α7 nicotinic acetylcholine receptor ; RIC-3

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) belong to a large and diverse family of acetylcholine-gated cationic ion channels. In mammals this family is composed of nine alpha subunits, whose N-terminus contains two disulfide linked vicinal cysteines enabling ligand binding, and seven non-alpha subunits. Most nAChR subunits assemble to form heteromeric receptors whereas α7 and α9 nAChR subunits can form homomeric receptors. Folding assembly and trafficking of mature nAChRs from the endoplasmic reticulum to the plasma membrane is a complex process which requires assistance from multiple cellular factors. Several proteins were shown to affect functional expression and properties of nAChRs including Resistance to Inhibitors of Cholinesterase-3 (RIC-3) and NACHO [1][2].

The nAChRs express widely and have multiple functions. In skeletal muscle nAChRs mediate excitation in the neuromuscular junction. Similarly, in the autonomic nervous system nAChRs are needed for excitatory synaptic transmission [3][4]. Functions of nAChRs, however, are not limited to synapses and to excitatory synaptic transmission. In the central nervous system (CNS) nAChRs have mostly modulatory roles, including a role in regulating release of neurotransmitters [5]. Moreover, these receptors are expressed in many non-excitable cells where they have been shown to affect migration, differentiation and proliferation [6].

2. α7 nAChR and the Cholinergic Anti-Inflammatory Pathway

α7 nAChR, a key player in the cholinergic anti-inflammatory pathway, was first identified as the α-bungarotoxin (α-BTX) binding receptor in the CNS [7]. This receptor has high permeability to calcium, likely to enable some of its effects on cellular functions [8]. In addition, α7 receptors are activated by ACh and by choline, a precursor and breakdown product of ACh as well as a breakdown product of membrane phospholipids. This enables α7-mediated responses to both targeted transmission and to localized tissue damage.

In immune cells α7 nAChR activation leads to phosphorylation and activation of Janus kinase 2 (JAK2) and to phosphorylation and nuclear entry of Signal Transducer and Activator of Transcription 3 (STAT3) which inhibits expression of pro-inflammatory cytokines. In parallel α7 nAChR activation reduces degradation of IkBα therefore inhibiting nuclear translocation of NF-kβ and the expression of pro-inflammatory cytokines. Interestingly, multiple lines of evidence suggest metabotropic activity of α7 nAChR in immune cells. Mechanisms enabling the immune modulating effects of α7 nAChR are reviewed in [9].

3. α7 nAChR and CNS Diseases

In mice loss of function mutations in subunits contributing to major CNS expressed nAChRs (α7, α4 and β2) have no easily discernible phenotypes [10]. But, in humans copy number variations of the gene encoding for α7 nAChR subunit (CHRNA7) are associated with brain diseases such as epilepsy or autism [11]. In addition, a 2bp deletion in of the gene encoding for a human-specific chimeric protein, CHRFAM7A, containing a duplicate of exons 5-10 of CHRNA7 , shows strong linkage to schizophrenia [12]. Instability of the genomic region harboring the CHRNA7 complicates its analysis, as

disease causing deletions encompassing this gene also cover additional genes. Since, heterozygosity for rare small deletions covering CHRNA7 alone is associated with similar phenotypes to those associated with larger deletions in this region it is likely that haplo-insufficiency of this gene is a cause for disease in humans [11], but some heterozygous carriers of CHRNA7 containing deletions are phenotypically normal. This confounding finding may be explained by incomplete penetrance of these deletions due to interactions with the genetic background or with the environment [11].

The CHRFAM7A chimeric protein, which is unique to humans, was shown to interact with and inhibit surface expression of α7 nAChRs [12][13]. This chimeric protein expresses in immune cells and its expression in these cells is regulated by LPS, nicotine and IL1β. Thus, this protein is likely to be a human specific regulator of the cholinergic anti-inflammatory pathway [12][13] .

Of note, involvement of α7 nAChR in neurodegenerative diseases may stem, at least partly, from its immunomodulatory effects, which in the CNS are likely to involve astrocytes and microglia [14]. As described above, α7 nAChR is expressed in glial cells and was shown to affect their activity.

4. α7 nAChR, RIC-3 and Multiple Sclerosis

The well-established role of α7 nAChR in the cholinergic anti-inflammatory pathway suggests that α7 may be involved in neuroinflammatory diseases, as reviewed by [9][14]. Several studies examined the cholinergic anti-inflammatory pathway in the animal model used for the study of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE) and in MS patients.

Using the EAE model it was shown that treatment with acetylcholine esterase inhibitors (AChEIs) reduced clinical severity and CNS inflammation intensity. This clinical amelioration was accompanied by reduction in lymphocyte proliferation and reduced inflammatory infiltrates to the spinal cord. These results suggest that AChEIs increase the concentration of extracellular ACh, rendering it available for interaction with a nicotinic receptor expressed on lymphocytes [15].

A marked improvement in EAE severity was found by treatment with rivastigmine (rivastigmine is a pseudo-irreversible AChEI) or with nicotine. These treatments also reduced proinflammatory respond, and improved CNS pathology [16].

Using direct activation of the α7 nAChR by specific ago-PAM, GAT107, in EAE also resulted in disease amelioration, accompanied by reduced neuroinflammtion and lower expression of proinflammatory cell markers [17].

Analysis in MS patients, showed increased responsiveness of peripheral blood mononuclear cells (PBMCs) to the anti-inflammatory effects of nicotine [18] and treatment with IFN-β led to reduce levels of pro-inflammatory cytokines and increased serum ACh levels; suggesting that circulating cytokines and ACh are co-regulated [19]. In addition, levels of ACh and of the enzymes responsible for its synthesis and degradation were altered in the serum of MS patients [19][20]. Analysis of NK cells from MS patients showed increased intracellular ACh levels [21]. Together, these changes suggest compensatory mechanisms occurring in MS patients aimed at reducing inflammation via enhanced activity of the cholinergic anti-inflammatory pathway.

RIC-3, like α7 nAChR is expressed in immune cells and this expression is regulated by pro-inflammatory stimuli, and its knockdown eliminates the anti-inflammatory effects of a α7 nAChR-specific agonist [22][23]. Thus, RIC-3 like α7 nAChR is likely to be involved in neuroinflammatory diseases. It is worth noting that genome wide association studies (GWAS) identified polymorphisms in the ric3 region in association with (MS) [24][25].

5. RIC-3 and α7 nAChRs in Neurodegenerative Diseases

RIC-3 was first characterized in C. elegans as an ER-resident protein that is needed for the maturation of multiple nAChRs but not for the maturation other ligand-gated ion channels [1]. Later analysis showed conservation of RIC-3 sequence and function during evolution [26]. Moreover, RIC-3 is required for heterologous expression of the α7 nAChR in non-neuronal mammalian cells [27].

Analysis of ric-3 loss-of-function mutants in C. elegans demonstrated reduced functional expression of multiple nAChRs in these mutants. Therefore, RIC-3 was suggested to promote surface expression of co-expressed nAChRs [1], but later analysis demonstrated both positive and negative effects of RIC-3 on co-expressed receptors depending on identity of the co-expressed receptor and on the expression system [26][28]. One explanation for these different effects is the finding that altering RIC-3-to-nAChR ratio shifts its effects from positive to negative [29][22].

RIC-3 was expected to promote expression of α7 nAChR in-vivo, as functional expression of α7 nAChR in non-neuronal cells requires co-expression with RIC-3 [27] and expression patterns of ric3 and the α7 nAChR subunit overlap [26]. However, knockout of ric3 did not significantly affect α7 nAChR expression in the brains of mice [30]. Nevertheless, its knockdown using siRNA-mediated silencing eliminated the anti-inflammatory effects of the α7 nAChR-specific agonist GAT107 [23]. This suggests cell-specific requirement of RIC-3 for functional expression of α7 nAChR.

While, neuroinflammation is causal for MS its role in AD and PD is less clear. Nevertheless, neuroinflammation is likely to contribute to progression of these diseases as well [31][32].

References

1. Halevi, S.; McKay, J.; Palfreyman, M.; Yassin, L.; Eshel, M.; Jorgensen, E.; Treinin, M. The C. elegans ric-3 gene is required for maturation of nicotinic acetylcholine receptors. EMBO J. 2002, 21, 1012–1020.
2. Gu, S.; Matta, J.A.; Lord, B.; Harrington, A.W.; Sutton, S.W.; Davini, W.B.; Bredt, D.S. Brain α7 Nicotinic Acetylcholine Receptor Assembly Requires NACHO. Neuron 2016, 89, 948–955.
3. Dani, J.A. Neuronal Nicotinic Acetylcholine Receptor Structure and Function and Response to Nicotine. Int. Rev. Neurobiol. 2015, 124, 3–19.
4. Richter, K.; Sagawe, S.; Hecker, A.; Küllmar, M.; Askevold, I.; Damm, J.; Heldmann, S.; Pöhlmann, M.; Ruhrmann, S.; Sander, M.; et al. C-Reactive Protein Stimulates Nicotinic Acetylcholine Receptors to Control ATP-Mediated Monocytic Inflammasome Activation. Front. Immunol. 2018, 9, 1604.
5. Dani, J.A.; Bertrand, D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu. Rev. Pharmacol. Toxicol. 2007, 47, 699–729.
6. Kurzen, H.; Wessler, I.; Kirkpatrick, C.J.; Kawashima, K.; Grando, S.A. The Non-neuronal Cholinergic System of Human Skin. Horm. Metab. Res. 2007, 39, 2191–2194.
7. Alkondon, M.; Albuquerque, E.X. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. J. Pharmacol. Exp. Ther. 1993, 265, 1455–1472.
8. Sequela, P.; Wadiche, J.; Dineley-Miller, K.; Dani, J.A.; Patrick, J.W. Molecular cloning, functional properties, and distribution of rat brain a7: A nicotinic cation channel highly permeable to calcium. J. Neurosci. 1993, 13, 596–604.
9. Treinin, M.; Papke, R.L.; Nizri, E.; Ben-David, Y.; Mizrachi, T.; Brenner, T. Role of the α7 Nicotinic Acetylcholine Receptor and RIC-3 in the Cholinergic Anti-inflammatory Pathway. Cent Nerv. Syst. Agents. Med. Chem. 2017, 17, 90–99.
10. Cordero-Erausquin, M.; Marubio, L.M.; Klink, R.; Changeux, J.P. Nicotinic receptor function: New perspectives from knockout mice. Trends Pharmacol. Sci. 2000, 21, 211–217.
11. Deutsch, S.I.; Burket, J.A.; Benson, A.D.; Urbano, M.R. The 15q13.3 deletion syndrome: Deficient α(7)-containing nicotinic acetylcholine receptor-mediated neurotransmission in the pathogenesis of neurodevelopmental disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry 2016, 64, 109–117.
12. Sinkus, M.L.; Graw, S.; Freedman, R.; Ross, R.G.; Lester, H.A.; Leonard, S. The human CHRNA7 and CHRFAM7A genes: A review of the genetics, regulation, and function. Neuropharmacology 2015, 96, 274–288.
13. de Lucas-Cerrillo, A.M.; Maldifassi, M.C.; Arnalich, F.; Renart, J.; Atienza, G.; Serantes, R.; Cruces, J.; Sánchez-Pacheco, A.; Andrés-Mateos, E.; Montiel, C. Function of partially duplicated human α77 nicotinic receptor subunit CHRFAM7A gene: Potential implications for the cholinergic anti-inflammatory response. J. Biol. Chem. 2011, 286, 594–606.
14. Tata, A.M.; Velluto, L.; D'Angelo, C.; Reale, M. Cholinergic system dysfunction and neurodegenerative diseases: Cause or effect? CNS Neurol. Disord. Drug Targets 2014, 13, 1294–1303.
15. Nizri, E.; Adani, R.; Meshulam, H.; Amitai, G.; Brenner, T. Bifunctional compounds eliciting both anti-inflammatory and cholinergic activity as potential drugs for neuroinflammatory impairments. Neurosci. Lett. 2005, 376, 46–50.
16. Nizri, E.; Irony-Tur-Sinai, M.; Faranesh, N.; Lavon, I.; Lavi, E.; Weinstock, M.; Brenner, T. Suppression of neuroinflammation and immunomodulation by the acetylcholinesterase inhibitor rivastigmine. J. Neuroimmunol. 2008, 203, 12–22.
17. Mizrachi, T.; Marsha, O.; Brusin, K.; Ben-David, Y.; Thakur, G.A.; Vaknin-Dembinsky, A.; Treinin, M.; Brenner, T. Suppression of neuroinflammation by an allosteric agonist and positive allosteric modulator of the α7 nicotinic acetylcholine receptor GAT107. J. Neuroinflamm. 2021, 18, 99.

18. Reale, M.; Di Bari, M.; Di Nicola, M.; D’Angelo, C.; De Angelis, F.; Velluto, L.; Tata, A.M. Nicotinic receptor activation negatively modulates pro-inflammatory cytokine production in multiple sclerosis patients. Int. Immunopharmacol. 2015, 29, 152–157.
19. Gatta, V.; Mengod, G.; Reale, M.; Tata, A.M. Possible Correlation between Cholinergic System Alterations and Neuro/Inflammation in Multiple Sclerosis. Biomedicines 2020, 8, 153.
20. Di Bari, M.; Reale, M.; Di Nicola, M.; Orlando, V.; Galizia, S.; Porfilio, I.; Costantini, E.; D’Angelo, C.; Ruggieri, S.; Biagioni, S.; et al. Dysregulated Homeostasis of Acetylcholine Levels in Immune Cells of RR-Multiple Sclerosis Patients. Int. J. Mol. Sci. 2016, 17, 2009.
21. Jiang, W.; Li, D.; Han, R.; Zhang, C.; Jin, W.N.; Wood, K.; Liu, Q.; Shi, F.D.; Hao, J. Acetylcholine-producing NK cells attenuate CNS inflammation via modulation of infiltrating monocytes/macrophages. Proc. Natl. Acad. Sci. USA 2017, 114, E6202–E6211.
22. Ben-David, Y.; Mizrachi, T.; Kagan, S.; Krisher, T.; Cohen, E.; Brenner, T.; Treinin, M. RIC-3 expression and splicing regulate nAChR functional expression. Mol. Brain 2016, 9, 47.
23. Ben-David, Y.; Kagan, S.; Cohen Ben-Ami, H.; Rostami, J.; Mizrahi, T.; Kulkarni, A.R.; Thakur, G.A.; Vaknin-Dembinsky, A.; Healy, L.M.; Brenner, T.; et al. RIC3, the cholinergic anti-inflammatory pathway, and neuroinflammation. Int. Immunopharmacol. 2020, 83, 106381.
24. Gourraud, P.A.; Sdika, M.; Khankhanian, P.; Henry, R.G.; Beheshtian, A.; Matthews, P.M.; Hauser, S.L.; Oksenberg, J.R.; Pelletier, D.; Baranzini, S.E. A genome-wide association study of brain lesion distribution in multiple sclerosis. Brain 2013, 136, 1012–1024.
25. Wang, J.H.; Pappas, D.; De Jager, P.L.; Pelletier, D.; de Bakker, P.I.; Kappos, L.; Polman, C.H.; Chibnik, L.B.; Hafler, D.A.; Matthews, P.M.; et al. Modeling the cumulative genetic risk for multiple sclerosis from genome-wide association data. Genome Med. 2011, 3, 3.
26. Halevi, S.; Yassin, L.; Eshel, M.; Sala, F.; Sala, S.; Criado, M.; Treinin, M. Conservation within the RIC-3 gene family: Effectors of nAChR expression. J. Biol. Chem. 2003, 278, 34411–34417.
27. Williams, M.E.; Burton, B.; Urrutia, A.; Shcherbatko, A.; Chavez-Noriega, L.E.; Cohen, C.J.; Aiyar, J. Ric-3 promotes functional expression of the nicotinic acetylcholine receptor alpha7 subunit in mammalian cells. J. Biol. Chem. 2005, 280, 1257–1263.
28. Lansdell, S.J.; Gee, V.J.; Harkness, P.C.; Doward, A.I.; Baker, E.R.; Gibb, A.J.; Millar, N.S. RIC-3 enhances functional expression of multiple nicotinic acetylcholine receptor subtypes in mammalian cells. Mol. Pharmacol. 2005, 68, 1431–1438.
29. Alexander, J.K.; Sagher, D.; Krivoshein, A.V.; Criado, M.; Jefford, G.; Green, W.N. Ric-3 promotes alpha7 nicotinic receptor assembly and trafficking through the ER subcompartment of dendrites. J. Neurosci. 2010, 30, 10112–10126.
30. Deshpande, A.; Vinayakamoorthy, R.M.; Garg, B.K.; Thummapudi, J.P.; Oza, G.; Adhikari, K.; Agarwal, A.; Dalvi, P.; Iyer, S.; Thulasi Raman, S.; et al. Why Does Knocking Out NACHO, But Not RIC3, Completely Block Expression of α7 Nicotinic Receptors in Mouse Brain? Biomolecules 2020, 10, 470.
31. Liddelow, S.A.; Guttenplan, K.A.; Clarke, L.E.; Bennett, F.C.; Bohlen, C.J.; Schirmer, L.; Bennett, M.L.; Münch, A.E.; Chung, W.S.; Peterson, T.C.; et al. Neurotoxic reactive astrocytes are induced by activated microglia.. *Nature* **2017**, *541*, 481–487, .
32. Mizrachi, T. , Vaknin-Dembinsky, A., Brenner, T., Treinin, M.; Neuroinflammation Modulation via α7 Nicotinic Acetylcholine Receptor and Its Chaperone, RIC-3. *molecules* **2021**, *26(20)*, :6139, .
