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NICOTINIC ALPHA7 ACETYLCHOLINE RECEPTOR: EXPRESSION, DISTRIBUTION AND FUNCTION IN NON-NEURONAL TISSUES

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ABSTRACT

Alpha7 acetylcholine receptor (α7AChR) belongs to the family of neuronal nicotinic acetylcholine receptors (nAChRs). It was thought that the receptor was expressed exclusively in central nervous system (CNS). However, that notion has changed, and it is evident that peripheral tissues and cells, outside the CNS, such as skeletal muscle and immune cells also express the receptor. The α7AChR is not only an ion-gated channel that facilitates calcium flow for cellular activity but also participates as a major constituent of the anti-inflammatory reflex in different organs and innate immune response to injury and inflammation. It has drawn attention by virtue of the potential for therapeutic manipulation to treat inflammation-related conditions both inside and outside the CNS. In this review the distribution of the α7AChR and its pharmacology outside the CNS in peripheral tissues are presented.

Keywords: alpha7 acetylcholine receptor; cholinergic anti-inflammatory pathway; immune cells; skeletal muscle.

1. INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are members of the super family of ligand-gated cationic channels, which also include GABA, 5-HT3 and glycine receptors, and facilitate neurotransmission and ion flow when stimulated by agonists with pluripotent downstream effects. The nAChRs are found in both the central nervous system (CNS) and peripheral nervous system (PNS) tissues. The focus of this review is the alpha 7 acetylcholine receptor (α7AChR), which forms either homomeric receptor consisting of five identical α7 subunits (Wang et al., 2003;Beissner et al., 2012;Baumann et al., 2019). In addition to the brain and spinal cord, the α7AChRs are also expressed in the autonomic sympathetic and parasympathetic ganglia, visceral and thoracic (visceral) organs, immune cells, skeletal muscle and skin (Table 1).

During the inflammation or tissue injury, the communication between CNS and peripheral tissues occurs through reflexes consisting of an afferent and efferent arc mediated by neurohumoral mechanisms (Waldburger and Firestein, 2010;Pavlov and Tracey, 2012;Steinberg et al., 2016). This review focuses on the bidirectional reflex arc mediated by the parasympathetic vagus nerve, which regulates and modulates physiological function and immune responses and of the visceral organs. The role of the sympathetic system is not discussed in this review. In several disease models, the visceral tissues and resident immune cells express α7AChRs, whose stimulation can lead to the immunomodulatory effects. For example, in celiac ganglia, vagus nerve communicates with splenic nerve, which passes signals in the spleen to stimulate α7AChR in macrophage by lymphocyte-secreted acetylcholine. (Tracey, 2002;Pavlov and Tracey, 2012;Tanaka et al., 2017). In addition to visceral organs, the skeletal muscles express α7AChRs during the fetal and neonatal stage (Fischer et al., 1999), and the keratinocytes in the skin also known to express α7AChRs (Ortiz and Grando, 2012).

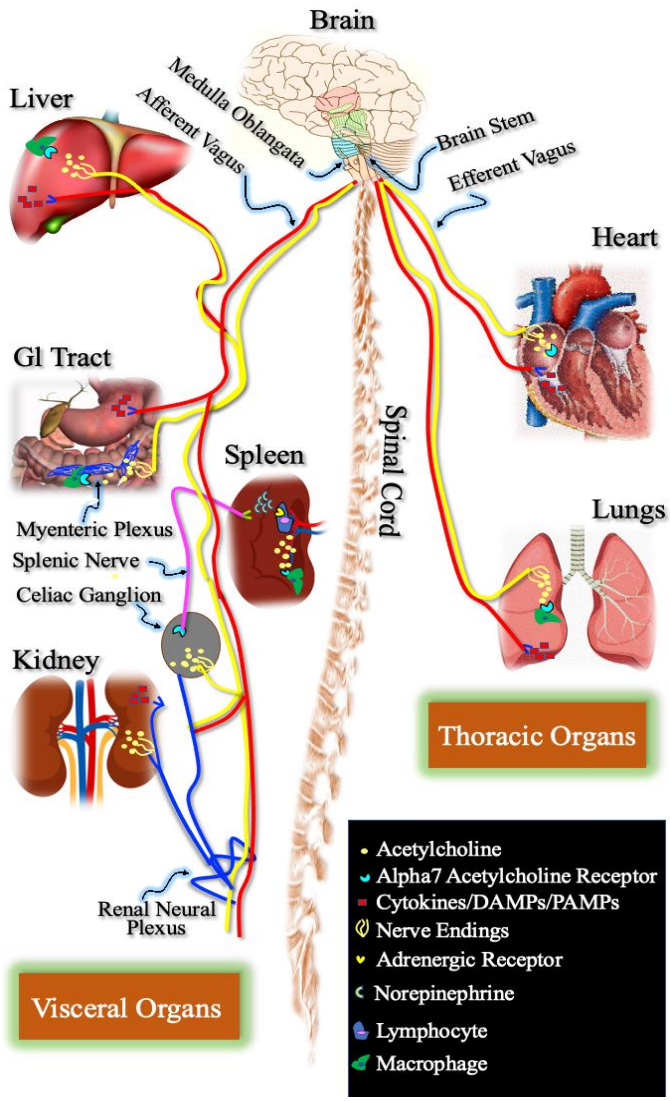

Fig. 1. The diagram illustrates the inflammatory reflexes of vagus nerve in the thoracic and visceral organs. The afferent and efferent vagus nerves activate excitatory and inhibitory pathway, respectively, and generate inflammatory reflex in the visceral (liver, GI tract, and spleen) and thoracic (heart and lung) organs. The inflammatory reflex occurs through parasympathetic and sympathetic nervous system and local neural plexuses. Vagus afferent arising from the viscera and thorax have their cell body in nodose ganglion that is projected into nucleus tractus solitarius (NTS) in the medulla oblongata of the brain stem. Sensitization of afferent vagus nerve by inflammatory cues such as cytokines/PAMPs/DAMPs passes the inflammatory signals to the NTS. The NTS interneurons then synapse with preganglionic vagal motor neurons of dorsal motor nucleus of the vagus (DMV), which, in turn, elicits anti-inflammatory response from efferent vagus nerve to release neurotransmitter, acetylcholine. This neurotransmitter binds to alpha7 acetylcholine receptor (α7AChR) in macrophages in the visceral and thoracic organs to trigger cholinergic anti-inflammatory mechanism by inhibiting proinflammatory mediators and reducing inflammation. In spleen, the inflammatory reflex is mediated by splenic nerve, which receives chemo-signal from efferent vagus nerve in the form of acetylcholine, which post-synaptic binds to the α7AChR in splenic nerve in celiac ganglion. The splenic nerve, in turn, releases norepinephrine to target adrenergic β2 receptor in the lymphocytes by evoking the release of acetylcholine, which subsequently bind to α7AChR in the macrophages to initiate cholinergic anti-inflammatory mechanism by forming an inflammatory arc.

Table 1: List of bio-distribution, molecular composition and gene expression of subunits of α, β, δ, γ and ε nicotinic acetylcholine receptors in multiple tissues of mouse

Gene Name	Gene Location	Base Pairs* (Mouse)	Amino Acids (Mouse)	Molecular Weight (kDa)[@]	Homology to Human (%)	Gene Expression in Tissues[#]
α1	2 C3; 2 43.76 cM	1368	456	52	96.1	SM
α2	14 D1; q4 34.36 cM	1536	512	60	81.3	B, L, SYG
α3	9 B; 9	1497	499	57	95.4	B, BD, DRG, E, ET, MNT, PLG, SC, SG
α4	2 H4; 2 103.54 cM	1887	629	70	94.1	B, E, ET, IE, S, K, PYG, I, O, SC, SG
α5	9 B; 9 29.84 cM	1314	438	51	90.8	AT, B, E, ET, FO, O, SG, T
α6	8; 8 A3	1482	494	53	86.0	B, E, M, PG
α7	7 C; 7 34.47 cM	1506	502	56	95.2	AT, B, BD, CB, ET, GT, H, IC, K, L, LG, SK, SM+
α8	Z	1536	511(Gg)	58	-	SYN[+]
α9	5 C3.1; 5 33.84 cM	1437	479	54	91.2	ET, IE, LG, SYG, TS
α10	7 E3; 7	1341	447	50	92.0	B, ET, H, LN, TE
β1	11 B3; 11 42.87 cM	1503	501	57	93.0	E, ET, EET, I, J, K, LG, LN, MYG, NX, P, PYG, S, SM, T, U
β2	3 F1; 3 39.19 cM	1503	501	57	97.6	B, DRG, E, ET, L, MYG, P, S, SC

β3	8; 8 A3	1392	464	51	92.6	B, E, ET, PYG
β4	9B; 9	1485	495	56	-	B, E, ET, IE, MYG, PLG
δ	1 D; 1 44.07 cM	1560	520	59	87.2	ET, IE, SK, SM
γ	1 D; 1 44.07 cM	1557	519	62	93.8	CT, ET, O, SM
ε	11 B3; 11 43.14 cM	1479	493	55	87.0	SM, T, TS

AT, Adipose Tissue; B, Brain; BD, Blood; CB, Carotid Body; CT, Connective Tissue; D, Dorsal Root Ganglion; E, Eye, ET, Embryonic Tissue; EET, Extraembyonic Tissue; FO, Fertilized Ovum; H, Heart; I, Intestine; IC, Immune Cells; IE, Inner Ear; J, Joint; K, Kidney; L, Liver; LG, Lung; LN, Lymph Node; M, Molar; MYG, Mammary Gland; MNT, Mature Nerve Terminal; NX, Nasopharynx; N, O, Ovary; P, Pancreas; PLG, Pineal Gland; PYG, Pituitary Gland; S, Spleen; SC, Spinal Cord; SG, Sympathetic Ganglion; SK, Skin; SM, Skeletal Muscle; SYG; Salivary Gland; SYN, synapse; T, Testis; TE, Tongue; TS, Thymus; U, Uterus. *Nucleotide sequence without stop codon, [@] predicted molecular weight, + expressed during embryonic stage, - not identified yet, Gg, *Gallus gallus* [#]source: http://www.ncbi.nlm.nih.gov/UniGene

α7AChRs (Ortiz and Grando, 2012). The ubiquitous expression of α7AChRs, as a part of cholinergic antiinflammatory mechanism, have made it a potential pharmacological target not only in neuronal cells but also in non-neuronal cells. The pharmacological importance of the α7AChR also led to generate several agonists and antagonists. This review presents distribution, expression and functions of α7AChR in non-neuronal cells.

2. α7AChR in inflammatory reflex

The vagus nerve is the tenth cranial nerve that originates on the either side of the nucleus tractus solitarius in the medulla oblongata of the brain stem (Wu et al., 2014;Noble et al., 2019). This nerve contains both afferent and efferent fibers which facilitate back and forth communication between brain and viscera (Fig. 1). It is considered as a major nerve of the parasympathetic system of the autonomic nervous system (Waldburger and Firestein, 2010). The parasympathetic nerve regulates physiological function and modulates peripheral inflammation by mounting a coordinate response to innate immune signals, which are generated during the invasion of pathogen and tissue injury (Pavlov and Tracey, 2012). The hepatic vagus nerve has been shown to regulate glucose production in the postabsorptive and postprandial state in the liver (Matsuhisa et al., 2000). The vagus nerve connects to the splenic nerve through the celiac ganglion. The release of norepinephrine (adrenergic neurotransmitter) by splenic nerve induces lymphocytes to secrete acetylcholine (cholinergic neurotransmitter), which in turn stimulates α7AChRs in macrophage and modulates immune function through an inflammatory arc (Tracey, 2002;Pavlov and Tracey, 2012).

3. α7AChR in immune cells

3.1. Macrophages

The α7AChRs in immune cells play a pivotal role in the activation of cholinergic anti-inflammatory pathway (Fujii et al., 2017;Ren et al., 2017), which is necessary to regulate cytokine release from immune cells such as macrophage, lymphocyte and neutrophil (Fig.2). Electrical stimulation of the vagus nerve inhibits increased levels of TNFa in wild type mice but fails to inhibit this cytokine in α7AChR-KO mice. The TNFa inhibition occurs through the stimulation of α7AChR by acetylcholine, which is released by the vagus nerve suggests that α7AChR is required for acetylcholine inhibition of TNFa release via cholinergic anti-inflammatory pathway (Wang et al., 2003). The activation of this pathway limits immune cells from excessive production of cytokines and subsequently attenuating inflammation. The α7AChR after activation triggers a signaling cascade, which involves many intracellular proteins such as Janus kinase-1/2 (JAK-1/2), signal transducer and activator of transcription-1/3/5 (STAT-1/3/5), phosphophatidyl inositol kinases (PI3K), protein kinase B (AKT) and NF-kB. These signaling molecules are involved in several proinflammatory/ anti-inflammatory pathways and participate in the regulation of inflammatory responses (Zdanowski et al., 2015;Li et al., 2020). In in vitro, lipopolysaccharide (LPS) upregulates expression of α7AChR in macrophages, as ~54 kDa protein (Fig. 3), with increased levels of TNFα release. However, treatment of LPS-induced macrophages with GTS-21, selective agonist of α7AChR, significantly decreases TNFα release. The involvement of α7AChR in inhibition of LPSinduced TNFα release is demonstrated by the treatment of LPS-induced macrophages with GTS- 21 after α7AChR knockdown in macrophages. The knockdown of α7AChR further elevates TNFα levels by nullifying the anti-inflammatory effects of GTS- 21 (Khan et al., 2012). Depending on stimuli, precursor macrophages (M0-type) polarize into subpopulation either to classically activated (M1- type) macrophages or alternatively activated (M2-type) macrophages by altering their properties and functions (Mantovani et al., 2013;Martinez and Gordon, 2014;Qin et al., 2017). As an example, it has been demonstrated that nicotine stimulates α7AChR to switch M0-type to M2-type macrophages that protect cells from endoplasmic reticulum stressinduced apoptosis. In contrast, the M2-type macrophages derived from α7AChR knockout mice show susceptibility to endoplasmic reticulum stressinduced apoptosis (Lee and Vazquez, 2013). The research involving macrophages indicates that the cholinergic anti-inflammatory activity associated with agonist stimulation of α7AChR has the capacity to polarize macrophages from M1 to M2 phenotype and vice versa and modulate cytokine profile both in sepsis as well as in sterile inflammation.

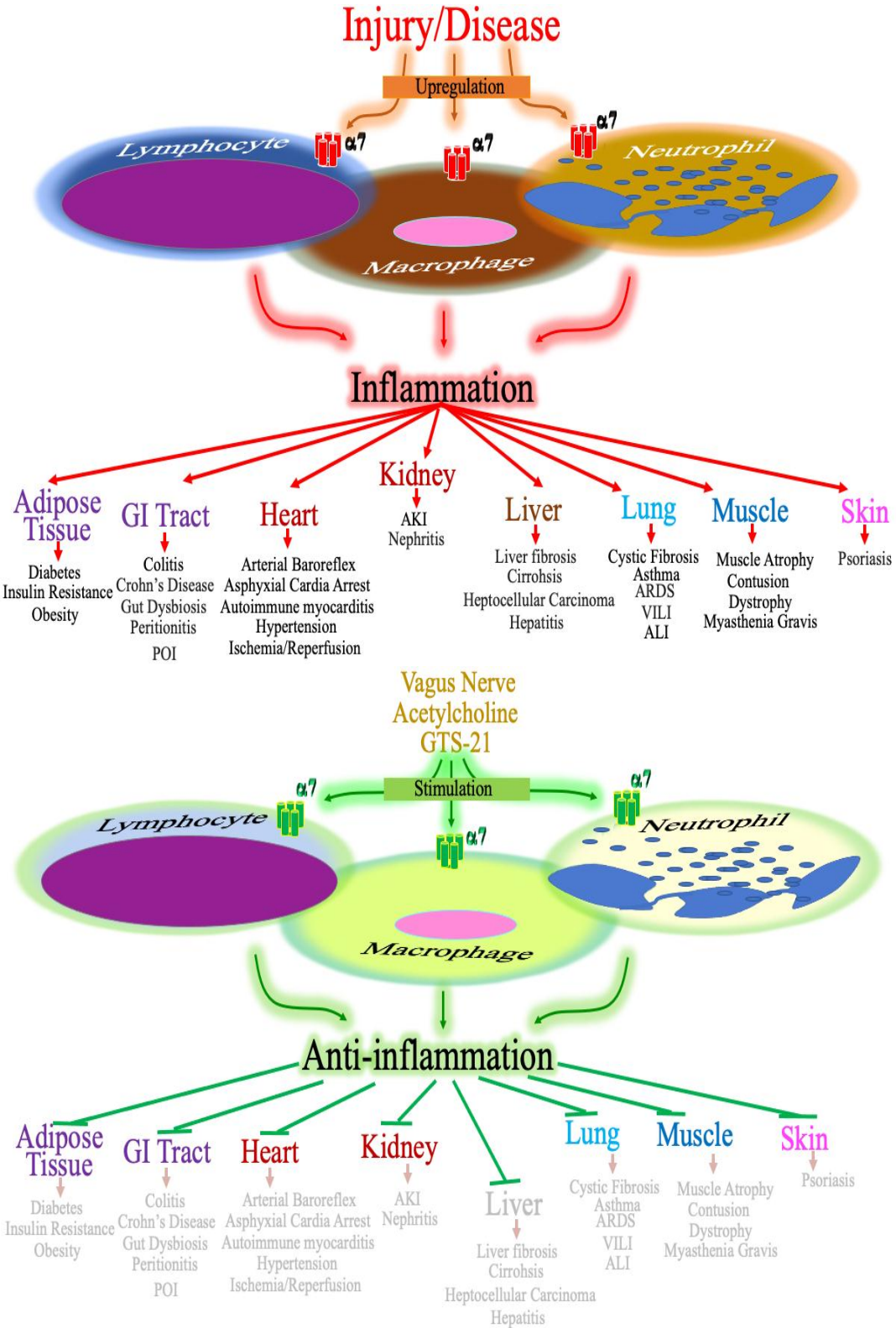

Fig. 2. Schematic diagram depicts the role of α7 acetylcholine receptor (α7AChR) in immune cells after inflammation. The immune cells such as macrophages, lymphocytes and neutrophils express α7AChR. The expression of α7AChR activates proinflammatory signaling pathways in immune cells, leading to sterile and/or septic inflammation and subsequently causing pathology in different organs (upper panel). Stimulation of inflammation-induced α7AChR by vagus nerve, or α7AChR endogenous agonist (e.g. acetylcholine) or exogenous agonist (e.g. GTS-21) triggers cholinergic anti-inflammatory pathway, which regulates the inflammation in different cells, tissues and organs. POI, postoperative ileus; ARDS, acute respiratory distress syndrome; VILI, ventilation-induced lung injury; ALI, acute lung injury.

3.2. Lymphocytes

Lymphocytes produce acetylcholine and contain the cholinergic machinery including choline acetyltransferase and acetylcholinesterase (Kawashima and Fujii, 2003;Fujii et al., 2017). The source of production of acetylcholine in the blood was unclear until it was found that acetylcholine in the blood originates from T-lymphocytes. The discovery of release of acetylcholine in the blood raised the assumption of expression of α7AChR in lymphocytes. The presence of α7AChR in Blymphocyte- derived cell lines is reported by Skok's

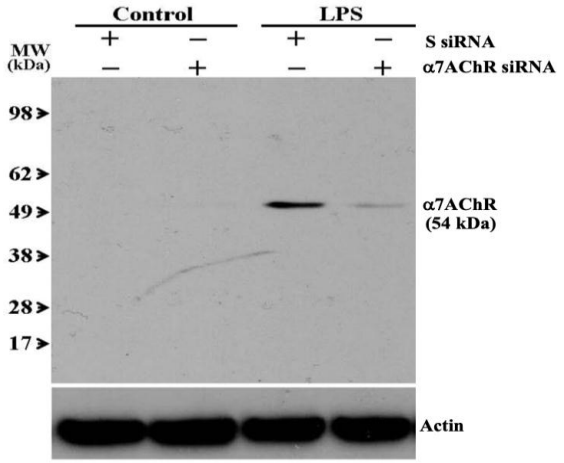

Fig. 3. Immunoblot analysis of α7AChR expression after siRNA knockdown in macrophages. The macrophages were treated with and without LPS. Immunoreactivity with anti- α7AChR antibody (ab 23832) confirms the antibody specificity by showing single band. MW indicates molecular weight. This image is reproduced from Khan et al., Shock, 2012.

group. Later, the same group has demonstrated the expression of α7AChR and secretion of acetylcholine in T lymphocytes (Skok et al., 2003;Skok et al., 2007;Koval et al., 2011;Koval et al., 2018). Stimulation of α7AChR by its agonist is critical in modulation of Ca2+ levels in T cells. The decrease of nicotine-induced increased Ca2+ levels is demonstrated by siRNA-mediated silencing of α7AChR in T cells (Razani-Boroujerdi et al., 2007). The finding of expression of α7AChR in lymphocytes suggest that α7AChR in the lymphocytes is also important for immune response.

3.3. Neutrophils

Neutrophils, like macrophages, also express α7AChR and get activated by various inflammatory mediators such as LPS. LPS-induced neutrophils also upregulate α7AChR, and their treatment with various α7AChR ligands modulates the neutrophil function (Giebelen et al., 2007b;Aomatsu et al., 2008;Lafargue et al., 2012). The stimulation of α7AChR by its ligand in neutrophils is typically associated with the inhibition of inflammation. For example, infiltration of leukocytes, including inflammatory neutrophils, into the airways of the lungs are significantly inhibited by GTS-21 stimulation of α7AChR after hyperoxia-induced lung injury (Sitapara et al., 2020), suggesting that α7AChR regulates neutrophils in inflammatory diseases.

4. α7AChR in visceral organs

Tissues in the visceral organs such as the heart, lungs, kidneys, gastrointestinal tract, liver and spleen including endocrine organ adipose tissue express α7AChRs (Wang et al., 2003; Wang et al., 2009; Filippini et al., 2012; Singh et al., 2014). The vagus nerve in the parasympathetic system of the autonomic nervous system regulates and modulates physiological function and immune responses of the visceral organs. Although a few reports show the presence of nicotinic α7AChR receptors in the heart, there is a meagre evidence that α7AChR is expressed by parenchymal cells (cardiomyocytes) in the heart. As an example, the α7AChR is shown to be largely expressed around the blood vessels of degenerated cardiomyocytes. The activation of α7AChR-mediated cholinergic anti-inflammatory pathway by acetylcholine protects cardiomyocytes, turning the heart more resistant to injury caused by ischemia and reperfusion (Gavioli et al., 2014). On the other hand, it may be possible that α7AChR in the heart may be originating from immune cells (Johansson et al., 2014).

The α7AChR is ubiquitously expressed in normal lung cells (Plummer et al., 2005). The plasma membrane of alveolar macrophages, neutrophils and bronchial epithelia of normal mouse lung type II cells and human alveolar epithelial type II cells have also shown strong immunoreactivity to α7AChR (Su et al., 2007). In lung injury models, the stimulation of α7AChR by nicotine and other selective agonists such as choline, GTS-21 and PNU282987 inhibit the lung injury with a marked decrease in excess lung water, lung vascular permeability, release of pro-inflammatory cytokines and neutrophil infiltration (Giebelen et al., 2007a;b; Su et al., 2007; Lafargue et al., 2012). In contrast, blockade of α7AChR with antagonist methyllycaconitine or genetic abrogation of α7AChR fails to decrease lung neutrophil recruitment and bacterial clearance. Instead, it reverses the beneficial effects of α7AChR agonists in mice with acid- or stroke-induced *pseudomonas aeruginosa* infected lung injury (Su et al., 2007; Lafargue et al., 2012). These recent reports indicate that the stimulation of α7AChR in alveolar macrophages and parenchymal cells in lungs contribute to the prevention of several kinds of lung injuries and diseases (Wang et al., 2019).

4.1. Gastrointestinal Tract

Gastrointestinal (GI) tract is highly innervated by the vagus nerve through the nerve plexuses, where α7AChRs are present in the enteric neuronal, glial and macrophages. Postoperative gastric ileus consists of two phases, an autonomic reflex and an inflammatory reflex (Stengel and Tache, 2011). In case of prolonged

inflammatory phase of postoperative gastric ileus, the activation of resident macrophages, which are present in the intestinal muscle layer, delay GI recuperation. Prevention of the development of postoperative ileus and reduction of intestinal inflammation are modulated by the stimulation of the vagus nerve mediated through α7AChR-dependent STAT3 signaling in intestinal macrophages or enteric glial cells (Bonaz et al., 2017). Dysfunction in the vagus nerve can change the amount of acetylcholine required to efficiently stimulate α7AChRs and activation of cholinergic anti-inflammatory pathway in the GI tract. This defect also can exaggerate other inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Expression of α7AChR along with other α3, α5, β2 and β4 subunits mRNA is detected by radioactive *in situ* hybridization in the myenteric plexus of the stomach, and small and large intestines (Garza et al., 2009). In addition to enteric glial cells, intestinal mesothelial cells are also shown to express α7AChR along with α9 and α10 subunits in this pathway. It is possible that the acetylcholine, which is released from enteric nerves can interact with the α7AChR present in the intestinal mesothelial cells in myenteric plexus neural network (Parrish et al., 2008; Mihara et al., 2017). In the event of intestinal ischemia and reperfusion, the increased oxidative stress, inflammation and apoptosis in the lung are attenuated by α7AChR activation (He et al., 2016). Costantini et al., have demonstrated that burn-induced intestinal permeability and limited histological gut injury are prevented by nicotine stimulation of α7AChR (Costantini et al., 2012). Treatment with α7AChR agonist PU282987 also shows protective effects against the apoptosis after radiation-induced GI injury (Chen et al., 2014). Collectively, this suggests that expression of α7AChR in enteric glial, macrophages and mesothelial cells can be used to pharmacologically target to prevent the gut inflammatory conditions and diseases.

4.2. Liver

Liver inflammation can result from elevated levels of pro-inflammatory cytokines, which are released from resident Kupffer cells. Hepatic stellate cells and liver sinusoidal endothelial cells, infiltrated macrophages and neutrophils also contribute to hepatic inflammation under various stimuli or diseases (Esser et al., 2014). Although expression of α7AChR is reported in Kupffer cells, other liver non-parenchymal cells express α7AChR in low abundance (Fabian-Fine et al., 2001; Gergalova et al., 2012; Hajiasgharzadeh et al., 2014; Nishio et al., 2017). In Kupffer cells, concanavalin A-induced autoimmune hepatitis is decreased through the inhibition of NF-kB signaling mediated by α7AChR cholinergic activity (Zhao et al., 2020). Using wild type and α7AChR knockout chimeric mice, it is demonstrated that LPS- and palmitic acid-induced NF-kB suppression in primary Kupffer cells from wild type mice is mediated by α7AChR. This effect is not seen in α7AChR knockout chimeric mice due to the absence of α7AChR gene. Nevertheless, α7AChR in the liver parenchymal cells (hepatocytes) contributes, independent of α7AChR activation in Kupffer cells, to the alleviation of insulin signaling through the inhibition of cytokines expression and c-Jun N-terminal Kinase, suggesting that not only Kupffer cells but also hepatocytes have α7AChR, which could be a potential target to protect liver during hepatic surgery, liver transplantation and other hepatic-related inflammatory disorders.

4.3. Kidneys

Kidneys are innervated with both afferent and efferent fibers of the renal plexus. There is no evidence whether the kidney is innervated with vagus nerve. Inoue et al., have shown that stimulation of vagus nerve in mice prior to ischemia-reperfusion injury significantly inhibits acute kidney injury through the attenuation of TNFα. In contrast, stimulation of vagus nerve in mice lacking α7AChR does not show protection against ischemia-reperfusion injury. Splenectomy inhibits the vagus nerve-induced anti-inflammatory effects. However, adoptive transfer of primed α7AChR splenocytes from the vagus nerve-stimulated mice protects the recipient mice with ischemia-reperfusion injury (Inoue et al., 2016; Inoue et al., 2017). Along these lines, vagus nerve-stimulated beneficial effects of acute kidney injury are abolished in α7AChR knockout as well as in splenectomized mice (Tanaka et al., 2019). This suggests that although the kidney is not directly innervated with vagus nerve, still α7AChR stimulation and its expression both are required for anti-inflammatory activity in the kidney. Several other investigations have suggested that α7AChR has mediatory role in the protection of the kidney as evident by its expression in proximal and distal tubes (Sadis et al., 2007; Yeboah et al., 2008; Li et al., 2011; Rezonzew et al., 2012). In another example, ultrasound treatment preserves kidney morphology and function through the stimulation of α7AChR-mediated cholinergic anti-inflammatory pathway (Gigliotti et al., 2013). These reports suggest that the expression and stimulation of α7AChR are necessary to protect against the inflammation of the kidneys.

4.4. Adipose Tissues

Dysregulation of adipose tissue is usually accompanied with abnormal production of adipokines, infiltration of macrophages (pro-inflammatory) and low-grade chronic inflammation in the adipose tissue and liver (Tateya et al., 2013). The α7AChR is expressed in various cell types, it is also believed that adipose tissue also expresses α7AChR. Recently, detection of α7AChR subunit in the adipose tissue has led to the investigation of α7AChR-mediated cholinergic mechanisms in low grade chronic inflammation-induced diabetes and obesity associated with insulin resistance (Wang et al., 2011; Cancello et al., 2012; Tateya et al., 2013). In a study of progression of metabolic disease, it is exhibited that when immune cells, lacking α7AChR, interact with metabolic tissues they further exaggerate metabolic derailment and contribute to the worsening of disease (Somm, 2014). In *ex vivo* experiment, the stimulation of α7AChR with specific agonist PNU282987 revealed a

significant increase in cholinergic anti-inflammatory activity in isolated human adipocytes from obese subjects (Cancello et al., 2012). The involvement of α7AChR in adipose tissues underlies the importance of cholinergic anti-inflammatory-driven regulation of obesity and insulin signaling.

4.5. Spleen

Spleen is a part of lymphatic system and consists of immune cells. Spleen not only abundantly express α7AChR but also a major source of serum TNFα, which contributes to the pathogenesis of sepsis (Vida et al., 2011). In the spleen, vagus nerve communicates with proximal side of the splenic nerve in celiac ganglion. On distal side, catecholaminergic fibers-containing splenic nerve innervates the spleen and secrete norepinephrine, which binds to b-adrenergic receptor in T lymphocytes. The release of acetylcholine from T lymphocytes, subsequently, stimulates α7AChR in splenic macrophage (Vida et al., 2011;Inoue et al., 2017). However, lymphocytes also express α7AChR but it is not clear whether acetylcholine released by T lymphocytes has an autocrine effects on α7AChR in T lymphocytes themselves in the spleen. It is also difficult to say that which neurotransmitter whether catecholamine from splenic nerve or release of acetylcholine from lymphocytes directly modulates the α7AChR in TNFα-releasing macrophages. Rosas-Ballina et al., describes a neural mechanism – cholinergic anti-inflammatory pathway – which controls systemic cytokine release, especially TNFα, via α7AChR after the electrical or pharmacological stimulation of efferent vagus nerve. The α7AChR agonist choline fails to reduce serum TNFα levels after splenic neurectomy, suggesting that splenic nerve requires stimulation of either vagus nerve or local stimulation of splenic nerve directly by

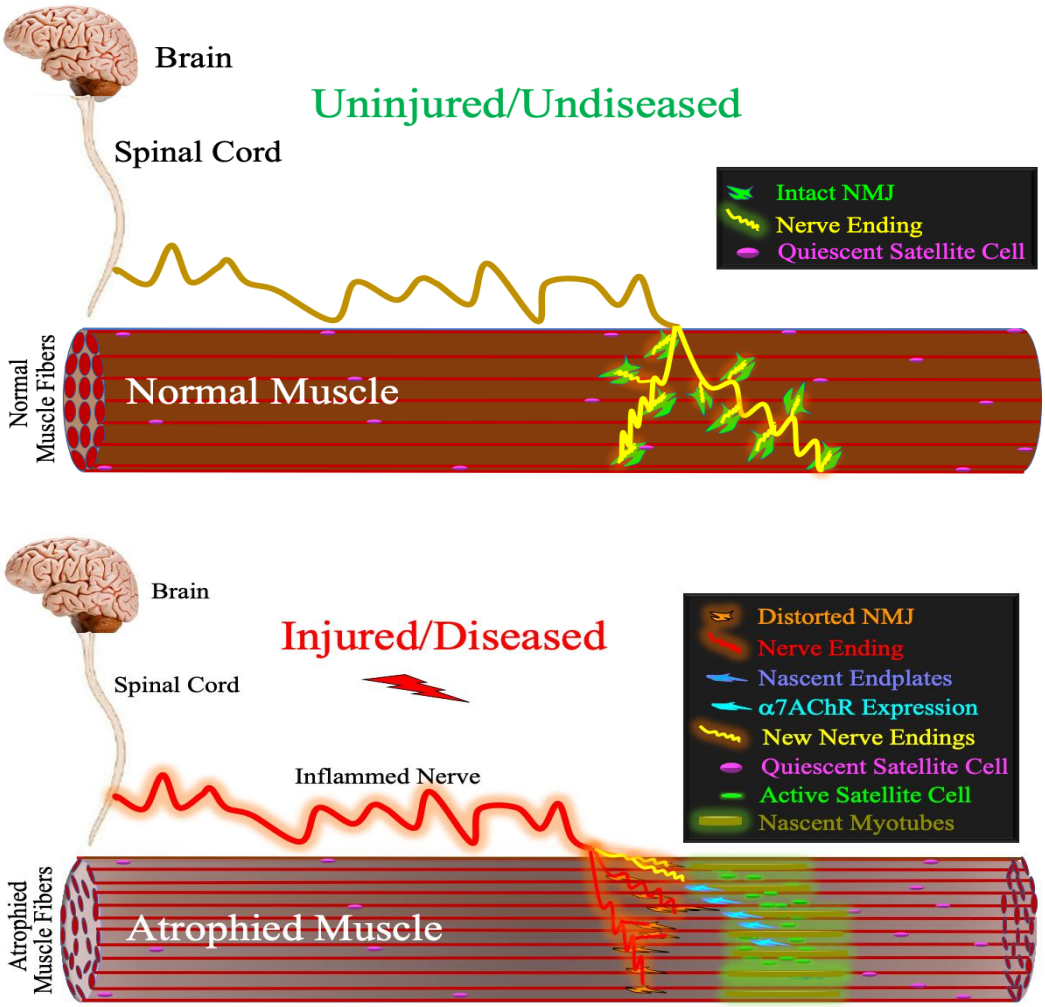

Fig. 4. Schematic diagram of normal and atrophied muscles. During the embryonic development and neonatal period, α7AChR is expressed pre- (nerve-side) and post-synaptically (muscle-side) to promote the formation of neuromuscular junction and to facilitate innervation of endplate on the muscle membrane. During this process, nerve endings from axon come into contact with endplates, bearing α1, β, δ, and ε AChR in neonatal, to form an intact neuromuscular junction (upper panel). In later stages, α7AChR disappears from normal (developed) muscles in adults. As opposite to the normal muscle (upper panel), the expression and reappearance of α7AChR are reported in atrophied

skeletal muscle (lower panel) after complete denervationor hindlimb immobilization (partial denervation)-induced muscle atrophy. During this muscle wasting process, the α7AChR reappears in the vicinity of damaged muscle fibers.

α7AChR agonist. They further demonstrate that splenic nerve stimulation but not the vagus nerve stimulation significantly inhibits serum TNFα levels in α7AChR knockout mice after endotoxemia. Seemingly, all three components; vagus nerve, splenic nerve and α7AChR are essential part of the anti-inflammatory mechanism in the spleen.

5. α7AChR in Skeletal Muscle

In the fetal and early neonatal stage, the skeletal muscles express α7AChRs together with heteromeric α1 and β1, δ, ε or γ receptor on postsynaptic side. In the late neonatal stage the skeletal muscles are devoid of α7AChRs (Fischer et al., 1999). Nonetheless, during diseased conditions or

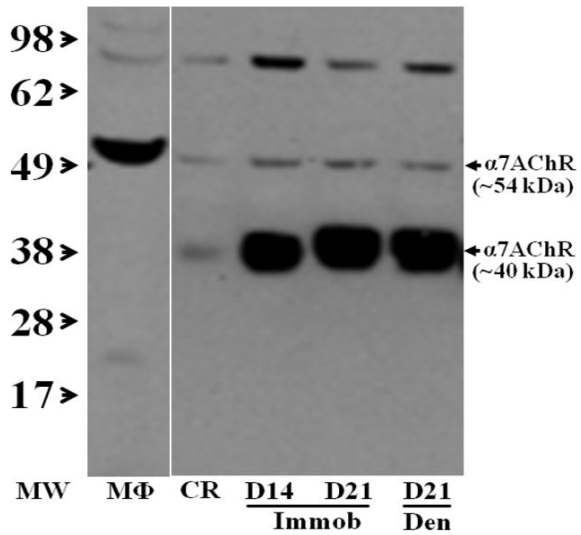

Fig. 5. Immunoblot analysis of expression α7AChR in skeletal muscle. The muscle protein extracts from immobilized and denervated hindlimb muscles are compared to contralateral hindlimb muscles of mice. Immunostaining with anti-α7AChR antibody (ab 23832; same antibody used to detect α7AChR in macrophages) produced multiple bands in muscle. The major band appeared at ~40 kDa with a minor band at ~54 kDa (also see refs. Fabian-Fine et al., 2001; Wells et al., 1998). The other top two bands may be a product of oligomerization or post-translational modification of α7AChR. Protein extracts from LPS-induced macrophages (MΦ) is used as positive control for α7AChR protein. MW, CR, D14 and D21 represent molecular weight and contralateral, day14 and day 21, respectively. Immob and Den indicates hindlimb immobilization and denervation, respectively.

disuse muscle atrophy α7AChR subunit reappears to innervate atrophying muscle. The α7AChR expression has not only been shown in skeletal muscle of human but also in mouse, rat and chick, Table 1, (Tsuneki et al., 2003;Martyn and Richtsfeld, 2006;Khan et al., 2014;Lee et al., 2014). In opposition to normal muscle, α7AChR is reexpressed in skeletal muscle after injury and during the period of pathogenesis of various muscle diseases, probably to re-innervate the dying muscle fibers, as depicted in Fig. 4 (Fischer et al., 1999;Lindstrom, 2003;Tsuneki et al., 2003;Leite et al., 2010;Lantzova et al., 2011;Fan et al., 2014;Kakinuma et al., 2014;Khan et al., 2014;Lee et al., 2014;Leite et al., 2014;Liu et al., 2014). In our studies, the analysis of expression of α7AChR protein in hindlimb immobilized muscle, using α7AChR polyclonal antibody (ab23832; Abcam, MA), exhibits that the same antibody, which is used to α7AChR in macrophages (Fig. 3), produces different results in skeletal muscle. In macrophages, the antibody detects only a single band of ~54 kDa (Fig. 3) whereas the same antibody with similar electrophoretic and protein transfer conditions shows multiple bands in hindlimb immobilized muscle. The disuse-induced muscle wasting after hindlimb immobilization or denervation showed a significant increase in α7AChR protein in skeletal muscle in immobilized hindlimb at 14 and 21 days, and denervated hindlimb at 21 days compared to respective contralateral limbs, controls, (Fig. 5). In the skeletal muscle, immunoblot blot analysis of α7AChR protein shows two bands, one at ~54 kDa and another at ~40 kDa. The band at ~40 kDa shows a greater expression than the band at ~54 kDa in skeletal muscle of immobilized hindlimb and denervated hindlimb. Additionally, higher molecular weight band of α7AChR protein is also seen in the blot. This band possibly may be resulted from either oligomerization or heavy glycosylation of α7AChR protein (Chen et al., 1998;Avramopoulou et al., 2004) because of the presence of abundant glucose, which has rapid turnover in skeletal muscle.

Other studies also report a relative increased expression of α7AChR protein either as a ~42 kDa or ~54 kDa band in the skeletal muscle at 14 days in models of sepsis, contusion and ischemia. As skeletal muscle cells are abundantly loaded with mitochondria and several other components such as proliferating satellite cells or nascent multinucleated myotubes, therefore, it is quite possible that the second form of truncated α7AChR (~42 kDa) might be originating from these components as an extrajunctional α7AChR (Fan et al., 2014;Kakinuma et al., 2014;Liu et al., 2014;Tian et al., 2015). Another possibility of appearance of truncated form of α7AChR in skeletal muscle can be attributed to several changes like RNA splicing, protein splicing and also a number of post-translational modifications such as glycosylation, phosphorylation, etc. (Tsunoyama and Gojobori, 1998), and also glycosidase enzyme activity, which deglycosylates glucose moieties from highly glycosylated proteins. More recently, in a rat model of skeletal muscle contusion, expression of α7AChR is reported in proliferated and differentiated satellite cells and regenerated multinucleated myotubes in the vicinity of wounded area (Tian et al., 2015). Undoubtedly, this suggests that skeletal muscle also express α7AChR not

only during embryonic development and neonatal period but also reappears in defective skeletal muscle and may be expressed in multiple forms.

6. α7AChR in Skin

In normal skin, α7AChR is expressed by keratinocytes (Ortiz and Grando, 2012). Unlike the visceral organs, the skin is not innervated by the vagus nerve, but there is a marked expression of α7AChR as well as local release of acetylcholine and choline in the skin (Osborne-Hereford et al., 2008). Recombinant version of non-canonical ligand/cholinergic peptides SLURP (secreted mammalian Ly-6/urokinase-type plasmogen activator receptor-related protein)-1 acts predominantly via α7AChR-coupled sedentary integrins (α2 and α3) during the epithelialization of cutaneous and oral wounds (Chernyavsky et al., 2012). Mouse deficient in α7AChR has elevated levels of IL-1β and IL-6 cytokines, which are accompanied with Ly6G+ neutrophils in the skin following topical application of croton oil (Gahring et al., 2010). Similar results of elevation of IL-1β and IL-6 are demonstrated with an additional evidence of SOCS3 (suppressor of cytokine signaling) as a possible mediator of inflammation in mouse skin after ultraviolet radiation treatment (Osborne- Hereford et al., 2008). An α7AChR-dependent mechanism is involved in the reduction of tropisetron-induced collagen synthesis in human dermal fibroblasts as well as maturation of keratinocytes and extracellular matrix turnover in the skin (Stegemann et al., 2013). Surprisingly, α7AChR-deficient mice have a transient delay in skin development during the first three weeks of life, which is attributed to decreased expression of apoptotic regulators. For example, the regulators such as Bad, Bax, and extracellular matrix proteins (collagen 1α1, elastin and metalloproteinase-1) are significantly decrease at both mRNA and protein levels in α7AChR-deficient mice (Arredondo et al., 2003). In a Toll-like receptor (TLR)-induced skin allograft transplantation, α7AChR also plays an important role in delaying skin allograft rejection and maintenance of tolerance by modulating IL-17 and IFNγ produced by alloreactive T cells in mice. Interestingly, the α7AChR knockout mice show the accelerated rejection of skin allograft compared to wild type recipient mice after the induction of TLR using TLR7 ligand, imiquimod. This suggests that α7AChR mediates regulation of alloreactivity and transplantation tolerance (Sadis et al., 2013) and it is also required for synchronizing biochemical events in moving keratinocyte during epidermal growth, skin repair and remodeling.

7. Agonists and Antagonists of α7AChR

The α7AChR is clinically relevant to several physiopathological disorders or diseases. Several α7AChR specific and partial agonists as well as antagonists have been used to stimulate or block α7AChR (Wang et al., 2003;Bitner et al., 2010;Lantzova et al., 2011;Khan et al., 2012;Vicens et al., 2013;Freedman, 2014;Bouzat et al., 2018;He and Shen, 2018;Verma et al., 2018). The α7AChR endogenous agonists, e.g., acetylcholine, choline and Lynx1, (Wang et al., 2003;Fu et al., 2012) and exogenous agonists, e.g., nicotine, GTS-21/DXMBA (a derivative of anabaseine compound from a nematode), PNU-282987, AR-R17779, ArlB and ABT-107 and aminobenzisoxazole compounds are currently used to pharmacologically target α7AChR (Kem, 2000;van Maanen et al., 2009;Lakhan and Kirchgessner, 2011;Khan et al., 2012;Parada et al., 2013;He and Shen, 2018). Among endogenous agonists, acetylcholine is the major vagus nerve product that specifically interacts with α7AChR, which is expressed in macrophages and other cell types to inhibit pro-inflammatory cytokine production in response to inflammatory stimuli (Wang et al., 2003). Nicotine is the long known exogenous agonist of AChR, hence the receptor named "nicotinic acetylcholine receptor". The research on nicotine involving α7AChR has generated mixed immunomodulatory responses because of it not only stimulate α7AChR but also other AChRs. The other specific agonist of α7AChR, GTS-21/DXMBA, has emerged as a potent investigational drug that has already reached Phase II clinical trials for Alzheimer's disease and infection-induced inflammation (Kem, 2000;Kox et al., 2011). GTS-21 has higher affinity than nicotine and implicated in the treatment of patients with Schizophrenia to improve cognizance (Cannon et al., 2013). Our group has shown that GTS-21 acts through the α7AChR to regulate LPS-induced inflammation (Khan et al., 2012) and burn-induced inflammation (Kashiwagi et al., 2017;Khan et al., 2017). Other agonists such as PNU-282987 and ARR17779 are also extensively studied and reported to be acting through the α7AChR to yield protective effects against several disease models (Vicens et al., 2013;Grandi et al., 2017;Liu et al., 2018). While ArlB and ABT-107 are discovered recently as specific agonists of α7AChR (Bitner et al., 2010;Hone et al., 2010;Malysz et al., 2010), the former is used as tracer in imaging of α7AChR in hippocampal neurons of wild type and α7AChR knockout mice (Hone et al., 2010) and the latter is described as a selective high affinity agonist of α7AChR in vitro and in vivo (Bitner et al., 2010;Malysz et al., 2010). Recently, c-11-labeled isotopomers and metabolites of GTS-21, iodinated α- conotoxin and Alexa Flour 546 conjugated ArlB, α- conotoxin peptide, are also employed to detect α7AChR (Kim et al., 2007;Hone et al., 2010;Kasheverov et al., 2011). As antagonists of α7AChR, α-bungarotoxin, α-conotoxin and methyllycaconitine are utilized in several studies (Malysz et al., 2010;Kasheverov et al., 2011;Khan et al., 2012). The α-bungarotoxin is a well-known antagonist of α7AChR that specifically and irreversibly binds to α7AChR in macrophages (Wang et al., 2003;Ghedini et al., 2008;Hone et al., 2010;Mikulski et al., 2010;Khan et al., 2012) and α7AChR and α1AChR at neuromuscular junction in the skeletal muscle (Khan et al., 2014). Most of the conventional, radio-labeled and fluorescent coupled agonists and antagonists are proving to be useful

tools to strategically design the pharmacological and pharmacokinetic studies involving α7AChRmediated signaling.

8. DISCUSSION AND CONCLUSION

A growing number of evidence shows that α7AChR, in addition to its integral role of in neuronal pathology in CNS, also plays an alternative role in the regulation of physiological, immunological and pharmacological functions in cells of non-neuronal origin in periphery. This receptor is now ubiquitously expressed in multiple tissues and cells. We and others show that α7AChR exists not only in neuronal cells but also in tissues of the visceral orangs, skeletal muscle, skin and immune cells. The α7AChR may exist with different molecular size in different cell types may be due to RNA or protein splicing or post-translational modifications. The omnipresence of α7AChR is intimately associated with cholinergic anti-inflammatory mechanisms, which has raised enthusiasm of many researchers to focus their research on clinical relevance of α7AChR to combat disorders and/or diseases ranging from neurological to physiological to inflammatory type by exploiting various α7AChR agonists.

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