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COMMENTARY

Application of Alpha7 Nicotinic Acetylcholine Receptor Agonists in Inflammatory Diseases: An Overview

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ABSTRACT Inflammatory disorders are characterized by the influx of immune cells into the vascular wall of veins and/or arteries in response to stimuli such as oxidized-LDL and various pathogens. These factors stimulate the local production of pro-inflammatory cytokines by macrophages and other cells that promote various inflammatory diseases such as atherosclerosis, Crohn's, Alzheimer's and diabetes. Numerous cytokines play a significant role in this process, though tumor necrosis factor (TNF) and various interleukins are thought to be among the most important regulators. These proinflammatory cytokines promote the above-described diseases by inducing endothelial cell dysfunction. In this brief commentary we will discuss some of the latest advances and discoveries in the treatment of these inflammatory diseases, making use of alpha7 nicotinic acetylcholine receptor (alpha7 nAChR) agonists.

KEY WORDS inflammation · alpha7 nicotinic acetylcholine receptor agonists · cholinergic anti-inflammatory pathway · Jak/STAT pathway

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CRUCIAL ROLE OF THE ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR IN THE CHOLINERGIC ANTI-INFLAMMATORY REFLEX

Recently, a novel discovered neural pathway has been described, which inhibits macrophage activation through parasympathetic outflow, termed the *cholinergic anti-inflammatory reflex*. This reflex pathway is activated by increased levels of pro-inflammatory cytokines produced in damaged tissues, which bind to and activate receptors on efferent vagal nerves, thus eliciting a central nervous system response to inflammation. Subsequent efferent vagal nerve activity leads to acetylcholine (ACh) release in the inflamed peripheral tissues. Convergence from many unrelated research areas has identified a primary role in health and disease for one of the most abundant subtypes of the nAChR, the alpha7 nAChR, which is expressed in the CNS, the autonomous nervous system, and the vascular system. This nAChR subtype is now emerging as a central regulator in processes ranging from cognitive processes through modulation of specific neurotransmitters, neuroprotection following various insults (e.g. chemical toxicity and beta-amyloid-induced cell death), normalization of sensory gating in schizophrenic patients and, more recently, inflammatory processes (1,2). Resident macrophages express the alpha7 nAChR, and activation of this receptor inhibits the production of pro-inflammatory cytokines (e.g. TNF, IL-1, IL-6, and high mobility group box 1 (HMGB-1)), but does not inhibit anti-inflammatory cytokine production (e.g. IL-10 and transforming growth factor-beta (TGF-beta)), thereby attenuating the local inflammatory response (1,2). Recent studies, with seminal contributions by the research team of Dr. Tracey, for example, have demonstrated that targeting pro-

inflammatory cytokines, such as TNF, IL-1 and IL-6, show promise in the treatment of inflammatory disorders, such as Crohn's, psoriasis, rheumatoid arthritis, asthma, sepsis, and diabetes (3–5). As such, nicotine, as well as the alpha7 nAChR agonists CAP55 and GTS-21, have been shown to be protective in models of sepsis, upon inhibiting local leukocyte recruitment and decreasing endothelial cell activation, effects that are mainly important in the microcirculation (4).

ROLE OF JAK/STAT IN THE ALPHA7 nAChR SIGNALING PATHWAY

Members of the Jak/STAT family pairs are tyrosine kinase enzymes (Jaks) and transcription factors (STATs) that rapidly transduce signals following the binding of cytokines, such as IL-6, as well as G-protein-coupled receptor agonists, such as angiotensin II and nicotine, to their cognate receptors. Upon receptor activation, the associated Jak protein phosphorylates residues in the SH2 domains of the cytoplasmic part of the receptor, thus initiating the recruitment of the STAT partner, which is subsequently phosphorylated by the Jak protein. The STAT transcription factor protein then homo- or heterodimerizes and translocates into the nucleus, where it binds to promoter regions of many target genes, as such, transactivating them. Although there are four members in the mammalian Jak family, Jak2 is the best studied one in nicotine-induced effects in vascular or neuronal tissues (6). Therefore, in this brief commentary, we will focus on the Jak2 tyrosine kinase.

Recent evidence has emerged showing that the CNS modulates the immune system through the reticuloendothelial system (RES). This CNS modulation is mediated through the vagus nerve, utilizing the major vagal neurotransmitter acetylcholine (ACh), which subsequently activates the alpha7 nAChR and the phosphotyrosine kinase Jak2. Signaling studies performed by our group have shown that upon activating PI3K (phosphatidylinositol 3-kinase) and AKT (protein kinase B), Jak2 represents an important part of a key cell survival pathway. Immunoprecipitation experiments moreover indicate that the alpha7 receptor and Jak2 interact directly (6). Furthermore, it has been shown in LPS-treated and control peritoneal macrophages that nicotine treatment leads to phosphorylation of STAT3, another key component of the cellular anti-apoptotic cascade. The nicotine-mediated phosphorylation is inhibited by the alpha7-selective antagonists alpha-bungarotoxin and methyllycaconitine, as well as by AG-490, a selective inhibitor of Jak2. Moreover, activation of the Jak2/STAT3 pathway protects from stroke in a mouse model (6).

Activation of the alpha7 nAChR results in neuroprotection against amyloid-beta, by means of activating a survival cascade initiated by the Jak2/PI-3K cascade and by inhibiting the pro-apoptotic pathway, upon inhibiting caspase 3 activation and activating the fragmentation of the DNA-repair enzyme poly(ADP-ribose) polymerase (PARP) (6). Additional studies have shown that the Ang II (angiotensin II) AT2 receptor performs a dominant-negative cross-talk, resulting in the complete neutralization of the alpha7 nAChR-Jak2 pro-survival cascade, by means of a SHP-1 phosphatase implicated in the tyrosine dephosphorylation of Jak2 (7).

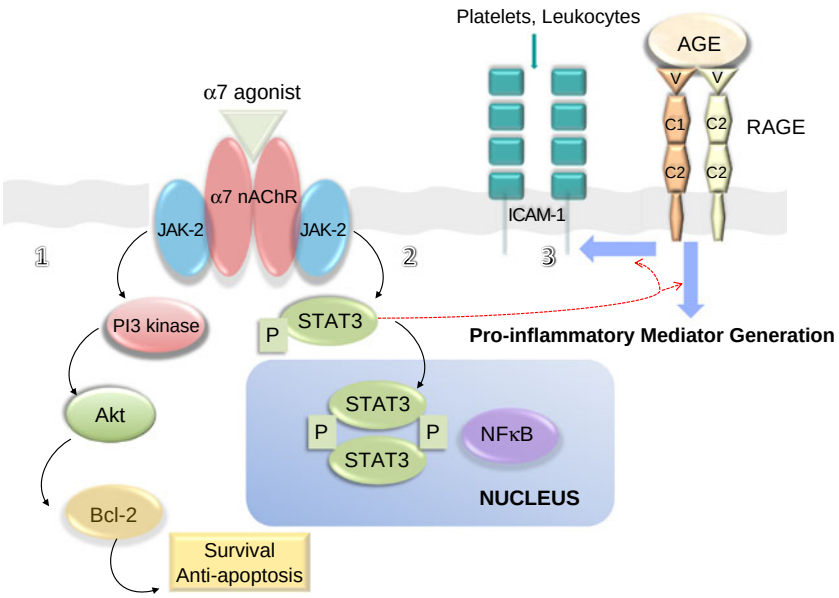

Fig. 1 Scheme of alpha7 nAChR-mediated anti-inflammatory effects. Agonists of the alpha7 nAChR induce Jak2 activation, which results in 1) PI3-kinase-mediated activation of AKT, providing a survival signal; 2) phosphorylation of STAT3 and inhibition of NF-kappaB and 3) inhibition of RAGE-mediated ICAM-1 upregulation and TNF/IFN-gamma generation. As a result of these activated pathways, both inflammation and apoptosis can be inhibited in alpha7 nAChR agonist-treated cells.

An alternative mechanism of action for the anti-inflammatory activity of alpha7 nAChR agonists was proposed in a recent study which reported that the inhibitory effect of nicotine on advanced glycation end-products-2 and -3 (AGE-2/3)-mediated monocyte activation, more specifically ICAM-1 upregulation and TNF/IFN-gamma production, can be inhibited by the specific alpha7 nAChR antagonist alpha-bungarotoxin. These findings thus suggest an interaction between the alpha7 nAChR and the receptor for advanced glycation end products (RAGE) (8). All of these studies thus support a crucial role for the interaction between alpha7 nicotinic agonists on the one hand and Jak2/STAT3 or RAGE on the other hand in the cholinergic anti-inflammatory pathway, ultimately leading to reduced NF-kappaB activation and reduced production of pro-inflammatory mediators (Fig. 1).

ALPHA7 nAChR AGONISTS AS ANTI-INFLAMMATORY AGENTS *IN VIVO*

In vitro observations on the role of the Jak2 pathway in the cholinergic anti-inflammatory pathway have been confirmed *in vivo*, e.g. in the modulation of the pro-inflammatory cascades involving TNF and interleukins, consistent with findings suggesting that alpha7 nAChR is an essential regulator of inflammation through modulation of pro-inflammatory cytokines and suppression of high mobility group box 1 (HMGB-1) secretion. A recent study clearly demonstrates the ability of the vagus nerve to modulate activity of the pro-inflammatory transcription factor NF-kappaB *in vivo* (9). One of our studies suggests a link between the neuroprotective pathway involving alpha7 nAChR-Jak2–Bcl-2 and the STAT3-NF-kappaB cascade, which is implicated in the decrease of pro-inflammatory mediator generation, including TNF, HMGB-1 and interleukins (1). Moreover, we recently demonstrated in a mouse model of type 2 diabetes that oral administration of TC-7020, a novel selective alpha7 nAChR agonist, reduced weight gain and food intake, reduced elevated glucose and glycated hemoglobin levels and lowered plasma levels of triglycerides and of TNF (3). Moreover, AG-490, a Jak2 inhibitor, also blocked these parameters, suggesting a linkage between the alpha7 nAChR and the Jak2/STAT3 pathways.

Recently, alpha7 nAChR$^{-/-}$ mice, upon treatment with LPS or upon infection with *E. coli*, were shown to develop more severe lung injury and to have higher mortality than corresponding alpha7 nAChR$^{+/+}$ mice (10). In this study, it was demonstrated that the immunomodulatory cholinergic alpha7 nAChR pathway of alveolar macrophages and neutrophils blocked both LPS- and *E. coli*-induced acute lung injury by reducing chemokine production and trans-alveolar neutrophil migration (10).

SIGNIFICANCE

In this commentary, we have summarized the role of the cholinergic anti-inflammatory pathway in the regulation of pro-inflammatory diseases. We have addressed some of the latest developments in the treatment of these diseases, linked to the anti-inflammatory effects of the alpha7 nAChR agonists, which seem to be mainly mediated through the Jak2/STAT3 pathway, ultimately leading to a reduction of NF-kappaB activation. The recent results obtained in this field will therefore extend our understanding of the effects of the cholinergic anti-inflammatory pathway on inflammation-related disorders like atherosclerosis, Alzheimer's disease, sepsis, and diabetes and will improve our knowledge of the mechanisms responsible for the beneficial effects of vagal nerve stimulation in treating theses diseases. This will provide further impetus for the promotion of new therapies for the prevention and treatment of these important diseases, for which no standard treatment exists to date.

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