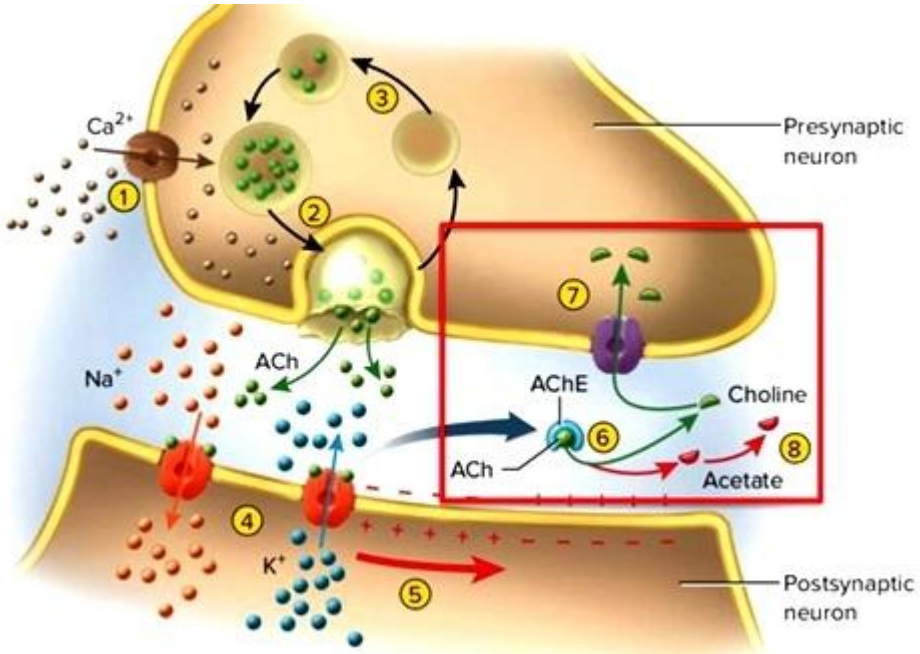
The SARS-CoV-2 spike (S) protein contains a neurotoxin-like region that has sequence similarities with the ectodomains of the rabies virus strains and snake neurotoxins (for example, α-bungarotoxin from snake *Bungarus* genera). In this work, the authors from the United States investigated whether the neurotoxin-like region of the SARS-CoV-2 S protein targets nicotinic acetylcholine receptors (nAChRs) and whether nicotine modifies this potential interaction.

AChRs are categorized as either metabotropic muscarinic (mAChRs) or ionotropic nicotinic acetylcholine receptors (nAChRs). Ionotropic neuromuscular and neuronal nAChRs are pentameric, cation-conducting channels that respond to the endogenous neurotransmitter acetylcholine (ACh) and are involved in signal transduction. Fast and non-selective opening of membrane-bound, excitatory cation channels results from their activation.

The nAChRs are composed of different homomeric or heteromeric combinations of 16 subunits, designated as α1-α7, α9, α10, β1-β4, δ, ε, and γ, based on sequence homology. The subunits α2-α7, α9-α10, and β2-β4 are expressed by neurons and are referred to as the neuronal nAChR subunits. The neuronal nAChRs are widely distributed throughout the central nervous system (CNS), particularly in the forebrain and brainstem. They have important physiological functions, including neurotransmitter release, mediation of cholinergic excitatory neurotransmission, synchronization of neuronal activity, and regulation of essential functions such as cognition, arousal, sleep, fatigue, anxiety, nutrition, central processing of pain, attention, and behavior (aggression, mood, and impulsivity). The composition of nAChR subunits determines the pharmacological and biophysical properties of these receptors. The most common nAChRs in the CNS, α4β2 and the α7 subtypes, are linked to hyperactivity, aggression, anxiety, and depression.

According to previous rodent and human studies, neuronal α7 nAChR plays a critical role in the modulation of aggressive behavior. It was demonstrated that the α7nAChRs were necessary for the anti-aggressive or ‘serenic’ effects of systemic administration of nicotine. It appears that hippocampal α7 nAChRs directly regulate aggression in mice, whereas the loss of their function leads to increased aggression. Some recent data linked the function of α7 nAChRs with depression.
https://www.sciencedirect.com/science/article/pii/S0969996125003390

In addition, nAChRs are present in numerous immune cells, including macrophages, T cells, dendritic cells, and B cells. ACh is involved in an anti-inflammatory response called the cholinergic anti-inflammatory pathway (CAP) that suppresses inflammation in the brain and peripheral tissues *via* the autonomic nerve fibers.



Acetylcholine neurotransmission

Changeux proposed initially that the neurotoxin-like region of the SARS-CoV-2 S protein interacts with nAChRs.
https://comptes-rendus.academie-sciences.fr/biologies/item/CRBIOL_2020__343_1_33_0/
Farsalinos et al. identified a “toxin-like” amino-acid sequence in the receptor binding domain (RBD) of the SARS-CoV-2 S protein (amino-acid *375–390*) which shows significant sequence homology with the neurotoxin homolog NL1, one of the many snake venom toxins interacting with nAChRs. They demonstrated that the main interaction occurs between the amino-acid sequence *381–386* of the RBD of the SARS-CoV-2 S protein and the amino-acid sequence *189–192* of the extracellular domain of the α9 nAChR, which is the core of the nAChR “toxin-binding site”. According to the authors, these findings strongly supported the hypothesis of the significant role of the nicotinic cholinergic system dysregulation in the pathophysiology of COVID-19. https://www.mdpi.com/1422-0067/21/16/5807

Later *in silico* study predicted that the neurotoxin-like region of the SARS-CoV-2 RBD interacts with high variability with models of the α7 and α4β2 nAChR. Agonists of nAChR are thought to stabilize a compact C loop conformation, whereas antagonists prevent C loop closure. In the α4β2 model, the neurotoxin-like region was unable to bind deeply into the orthosteric-binding site, keeping the C loops of the receptor in an open conformation. Nevertheless, in the α7 nAChR model of nAChR, the neurotoxin-like region showed multiple modes of the C loop, ranging from an open conformation to a semiclosed structure if it moved deeper into the binding pocket.
https://www.sciencedirect.com/science/article/pii/S0006349521001466

Other studies have also discussed the role of cholinergic deficiency in various COVID-19 syndromes. Recent animal study has shown that inoculation of the S1 subunit of the SARS-CoV-2 S protein in the olfactory cavity resulted in increased apoptosis of the olfactory system, brain inflammation, and reduced ACh levels in the mouse brain. The administration of donepezil, a central cholinergic agent and acetylcholine esterase inhibitor, normalized inflammatory cytokines that were previously elevated and mitigated enhanced apoptosis in the olfactory bulb.
https://discovermednews.com/s1-protein-causes-brain-inflammation-and-decreases-the-acetylcholine-levels/

About the study

The authors employed two-electrode voltage clamp electrophysiology to investigate the interaction of subtypes α7, α4β2, α3β4, and α3β2 of nAChRs with SARS-CoV-2 glycoprotein peptide (ScoV2P), which is the neurotoxin-like region of SARS-CoV-2, and the SARS-CoV-2 ectodomain (SCoV2ED). These nAChRs were chosen because they are expressed in the target tissues of SARS-CoV-2, such as the nose, lungs, CNS, and some immune cells. The researchers hypothesized that SCoV2P and SCoV2ED will antagonize the chosen subtypes of nAChR, and that nicotine will enhance this inhibitory effect.

Live-cell confocal microscopy was used to confirm that SCoV2P interacts with nAChRs in transfected neuronal-like N2a and human embryonic kidney 293 (HEK293) cells.

α7 nAChR

Results

High concentrations of ScoV2P, a neurotoxin-like region of SARS-CoV-2, moderately inhibited the α4β2, α3β4, and α3β2 subtypes of nAChRs.

The ScoV2P exerted an opposing, bimodal, and concentration-specific effect on the α7 nAChR. A high concentration of ScoV2P significantly inhibited α7 nAChR and reduced ACh potency, whereas low concentrations potentiated ACh-induced currents. In addition, the results showed that SCoV2P can bind to α7nAChRs in different orientations and exerts its dual action- potentiation or inhibition of the α7 nAChR through an allosteric mechanism. SCoV2P did not have any similar allosteric actions on heteromeric nAChRs. At high concentrations, ScoV2P inhibited the ACh-induced currents of α4β2, α3β2, and α3β4 nAChRs with some isoform-specific effects.

Confocal imaging confirmed that ScoV2P interacts with α7 nAChRs expressed on the cell surface.

These findings revealed an antagonizing and selective effect of ScoV2P on nAChRs, with a high preference for the α7 subtype. This confirms that the α7nAChR is a target for the neurotoxin-like region of SARS-CoV-2. These results also suggest that SCoV2P binding to α7 nAChRs can prime receptors for agonist activation.

Importantly, pretreatment with nicotine enhanced the modulation of α7 nAChR responses by SCoV2P and ScoV2ED, resulting in increased potentiation *via* a mechanism that resensitizes desensitized nicotinic receptors. In the absence of nicotine, the α4β2, α3β2, and α3β4 subtypes of nAChRs were minimally inhibited. Pretreatment with 200 nM of nicotine enhanced the inhibition of currents of (α4β2)2α4 and (α3β2)2α3 nAChRs by ScoV2P and made the inhibition of currents of (α3β2)2α3 nAChR similar to the inhibition of α7 nAChR (40%).

According to the authors, nicotine pretreatment broadened the effects of SCoV2P by inhibiting not only the α7 subtype of nAChRs but also α4β2 and α3β2 subtypes. Accordingly, the ability of the SARS-CoV-2 neurotoxin-like region to resensitize desensitized α7 nAChRs in tobacco users could further activate their cholinergic anti-inflammatory response, allowing higher levels of viral replication.

Conclusion

These results demonstrated that nAChR subtypes interact with the neurotoxin-like region of SARS-CoV-2, confirming that the α7 nAChR is a target for SARS-CoV-2. Low concentrations of SCoV2P and SCoV2ED positively modulated ACh-mediated currents *via* the facilitation of α7 nAChRs transition to the active conformation. In contrast, higher concentrations of both ScoV2P and ScoV2ED switched the modulation activity to inhibition.

Interestingly, intracerebroventricular administration of PNU282987, an agonist of α7 nAChRs, a week after the intranasal inoculation of the SARS-CoV-2 S1 protein, normalized or increased the expression of inflammatory cytokines in the brains of S1 mice. These results showed the involvement of α7 nAChRs in the cholinergic anti-inflammatory pathway and the connection of brain inflammation with a disrupted cholinergic anti-inflammatory pathway. https://discovermednews.com/s1-protein-causes-brain-inflammation-and-decreases-the-acetylcholine-levels/

The α7 nAChRs are present in numerous immune cells, including macrophages, T cells, dendritic cells, and B cells. The activation of α7 nAChRs in the cholinergic antiinflammatory pathway suppresses the production of proinflammatory cytokines. The recombinant trimeric rabies virus glycoprotein binds to α7 nAChRs expressed on monocyte-derived macrophages, activating the cholinergic anti-inflammatory pathway. This led to suppressed function of macrophages as T-cell activators, and showed that rabies virus could induce an anti-inflammatory state in human macrophages through interactions with α7 nAChR. Also, the

dorsal root ganglion, which is believed to be a crucial bridge allowing the rabies virus to pass from the periphery into the CNS, expresses a variety of neuronal nAChR subtypes, including α7. https://doi.org/10.1016/j.heliyon.2022.e104

The authors concluded that understanding how the SARS-CoV-2 neurotoxin-like region affects different nAChR subtypes and associated isoforms provides an understanding of COVID-19 pathophysiology, which very likely facilitates the development of targeted therapeutics.

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