Review

α7- and α9-Containing Nicotinic Acetylcholine Receptors in the Functioning of Immune System and in Pain

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Abstract: Nicotinic acetylcholine receptors (nAChRs) present as many different subtypes in the nervous and immune systems, muscles and on the cells of other organs. In the immune system, inflammation is regulated via the vagus nerve through the activation of the non-neuronal α7 nAChR subtype, affecting the production of cytokines. The analgesic properties of α7 nAChR-selective compounds are mostly based on the activation of the cholinergic anti-inflammatory pathway. The molecular mechanism of neuropathic pain relief mediated by the inhibition of α9-containing nAChRs is not fully understood yet, but the role of immune factors in this process is becoming evident. To obtain appropriate drugs, a search of selective agonists, antagonists and modulators of α7- and α9-containing nAChRs is underway. The naturally occurring three-finger snake α-neurotoxins and mammalian Ly6/uPAR proteins, as well as neurotoxic peptides α-conotoxins, are not only sophisticated tools in research on nAChRs but are also considered as potential medicines. In particular, the inhibition of the α9-containing nAChRs by α-conotoxins may be a pathway to alleviate neuropathic pain. nAChRs are involved in the inflammation processes during AIDS and other viral infections; thus they can also be means used in drug design. In this review, we discuss the role of α7- and α9-containing nAChRs in the immune processes and in pain.

Keywords: anti-inflammatory pathway; chronic pain; α-conotoxins; Ly6/uPAR proteins; Lynx1; nicotinic acetylcholine receptors; SLURP-1; viral infection

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels belonging to the family of Cys-loop receptors [1]. Structural studies of nAChRs started with the muscle-type receptor from the electric organ of ray; this receptor consists of five subunits (two α1, β1, γ and δ) and is most closely related to the muscle receptors of mammals. Our review mainly deals with "non-neuronal" nAChRs, which are built from the same subunits as those in the nervous system (neuronal nAChRs) but are localized in other tissues. Nine α (α2–α10) and three β (β2–β4) subunits are currently known among these neuronal and non-neuronal subunits. Some subunits (α7, α8, α9) can be part of both homopentameric (α7, α9) and heteropentameric receptors (α7α8, α7β2, α9α10), the remaining subunits form heteropentameric channels, for example, α4β2 and α4α6β2β3.

The ligand-binding sites of all nAChRs always involve α-subunits, which have a functionally important disulfide bond formed by two vicinal cysteines in the so-called loop C, which is one of the main elements of the orthosteric binding site for agonists and competitive antagonists of all nAChRs. This disulfide is characteristic only for α-subunits, in contrast to the Cys-loop, which is present in all nAChR subunits. Orthosteric binding sites are located on the surface between the α-subunit and its adjacent subunit.

Since nAChRs have an important role in the functioning of various systems in organisms, including the immune system, and are involved in a number of pathological processes, including pain, they are considered to be promising targets for next-generation

therapies. Some nAChR ligands have become medicines, e.g., the anti-smoking drugs cytisine and varenicline are already on the market; however, the treatment of immune system disorders or pain with nAChR ligands is still a challenging task. The problem is that there is a number of different nAChR subtypes, and they are present in many systems and organs; thus, their activation or inhibition may result in adverse effects. This problem can be solved by the application of highly selective ligands that affect only certain nAChR subtype or subtype populations in a particular organ. Among the various subtypes, $\alpha 7$ nAChR has been found to be deeply involved in immune system function, and $\alpha 9\alpha 10$ nAChR has been considered to be involved in pain alleviation. Two main hypotheses are considered in this review:

- nAChRs of $\alpha 7$ subtype are involved in anti-inflammatory pathways;
- $\alpha 9\alpha 10$ nAChRs participate in pain relief mechanisms.

Many of the data available in the literature are in favor of these hypotheses and are considered in this review. We try to provide current information on the compounds affecting the $\alpha 7$ and $\alpha 9\alpha 10$ nAChRs and discuss the animal toxins of α-conotoxins and α-neurotoxins, which are so far the most selective and efficient ligands (in general, inhibitors) of these receptor subtypes. While there is quite a lot of information available on the structure and activity of these compounds, and they are considered promising candidates for drug design, much more needs to be undertaken to turn them into medicines. The possible directions for this transformation are also discussed in this review.

Heteromeric and homomeric nAChRs, containing $\alpha 7$ and $\alpha 9$ subunits, have recently attracted the greatest research interest due to the revealed participation of these receptors in immune processes and pain, as well as due to their ability to be recognized by viruses. However, understanding the molecular mechanisms of the participation of these receptors in such processes is impossible without knowledge of their spatial organization.

2. Spatial Structure of Nicotinic Receptors and Their Complexes with Various Ligands

It was a long way to the elucidation of the spatial structure of nAChRs, which started with the low-resolution X-ray structure of the nAChR from the electric ray [2], followed by low-resolution cryo-electron microscopy [3], while the next important stage was obtaining the high-resolution X-ray structure of the water-soluble acetylcholine-binding protein (AChBP) [4], which is an excellent model of the ligand-binding domains of not only nAChRs but all other Cys-loop receptors. This structure helped to establish the cryo-electron microscopy structure of the *Torpedo marmorata* nAChR, with a resolution of 4.5 Å [5]. This was then followed by the X-ray structures of the microbial Cys-loop receptors ELIC and GLIC, and only after this were several X-ray structures established for a couple of neuronal nAChRs and for their complexes with agonists and antagonists. What concerns the topics of our review, among which is brief information on such fine tools for the nAChR research, as snake venom protein neurotoxins (Figure 1A) and neurotoxic peptides α-conotoxins from *Conus* marine snails, it should be noted that there are the structures of α-bungarotoxin (a long-chain α-neurotoxin) with the *Torpedo* and $\alpha 7$ nAChR [6] (Figure 1B), as well as of a short-chain snake venom α-neurotoxin (which does not inhibit neuronal nAChRs) bound to the *Torpedo* receptor [7].

In the structure presented in Figure 1B, the upper part of the receptor over the membrane shows the extracellular ligand-binding domain (ECD) that includes the orthosteric ligand binding sites for agonists (acetylcholine (ACh), nicotine, synthetic compounds) and for such competitive antagonists, such as snake venom three-finger α-neurotoxins or α-conotoxins. In each receptor subunit, the intramembrane portion contains four transmembrane fragments; the major role in the organization of the channel is played by five membrane helices M2 from each of the five subunits. The lower part, the cytoplasmic domain, in all of the mentioned X-ray structures was not completely resolved, although this portion should be of functional importance for further signal transduction. For example, it is assumed that in the intracellular domain of $\alpha 7$ nAChR, there are binding sites for some proteins that are capable of triggering intracellular signaling cascades, which explains the

metabotropic pathway of nAChR signaling in non-excitable cells, such as the cells of the immune system [8]. The first data on the structure of the cytoplasmic domain have been recently obtained through the use of ^{1}H-NMR spectroscopy [9]. Several X-ray structures of complexes of various ligands with recombinant nAChR ECDs have also been obtained, for example, α7 ECD with α-bungarotoxin (Figure 1C).
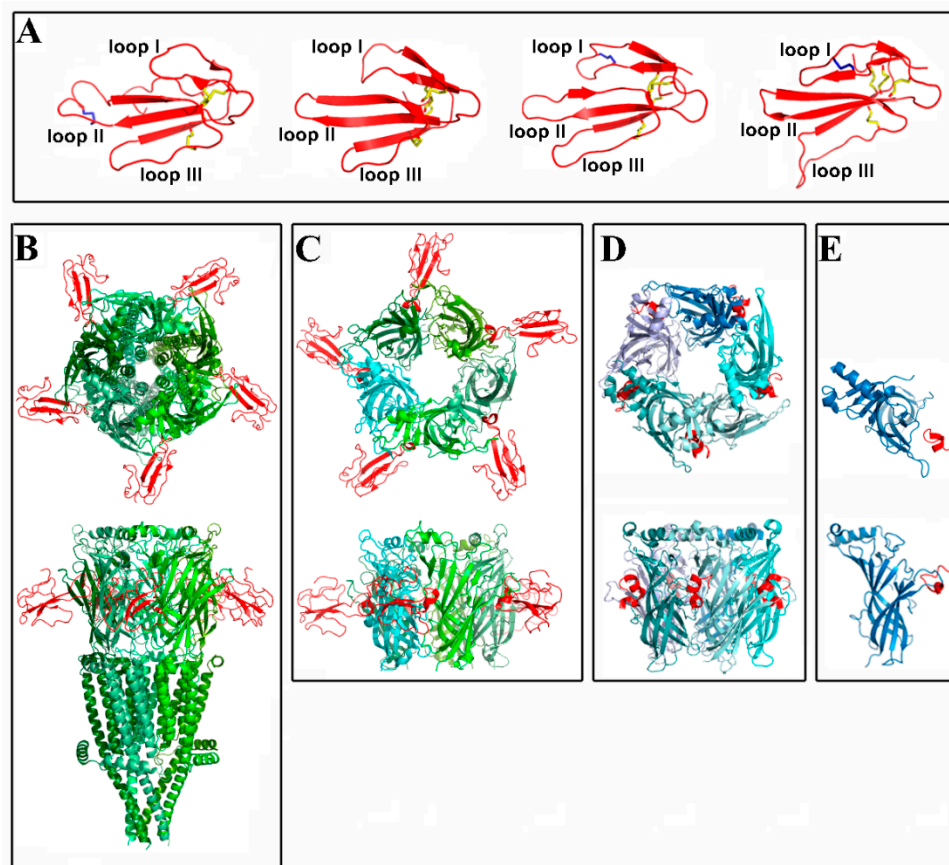

Figure 1. The ribbon presentation of spatial structures of three-fingered toxins as well as different complexes of the target ligand from NMR, X-ray or cryo-EM studies. (**A**) From left to right: X-ray structures of long-chain α-cobratoxin from *Naja kaouthia* (PDB ID: 2CTX) and short-chain erabutoxin-a from *Laticauda semifasciata* (PDB ID: 5EBX) as well as NMR structures of non-conventional candoxin from *Bungarus candidus* (PDB ID: 1JGK) and human water-soluble Lynx1 protein (PDB ID: 2L03). Four "core" disulfides are shown in yellow and the fifth disulfide, if any, are in blue. Three loops are indicated in all cases. (**B**) Top and side view of the cryo-EM structure of pentameric construct based on human α7 nAChR complexed with α-bungarotoxin (PDB ID: 7KOO). Identical subunits of the receptor are shown with different colors of green for clarity, and five toxins are in red. (**C**) Top and side view of the X-ray structure of pentameric chimera based on human α7 nAChR extracellular domain (ECD) and *Lymnaea stagnalis* AChBP complexed with α-bungarotoxin (PDB ID: 4HQP). Identical protomers are shown with different colors of green for clarity, and five toxins are in red. (**D**) Top and side view of the X-ray structure of *Aplysia californica* AChBP complexed with α-conotoxin PnIA analogue (PDB ID: 2BR8). Identical protein protomers are shown with different colors of blue for clarity, and five conotoxins are in red. (**E**) Top and side view of the X-ray structure of monomer of human α9 nAChR ECD (in blue) complexed with α-conotoxin RgIA (in red) (PDB ID: 6HY7).

3. Snake α-Neurotoxins and Ly6/uPAR Proteins as Research Tools and Potential Drugs

α-Bungarotoxin, a representative of three-finger snake venom α-neurotoxins, has played a crucial role in the isolation and characterization of the first individual muscle-type nAChR. α-Bungarotoxin and other so-called α-neurotoxins (Figure 1A) are still well-recognized tools in research on nAChR, helping to identify their certain subtypes in tis-

sues and clarify their involvement in different cell mechanisms, including immunological processes and pain perception. However, they do not allow for the differentiation of certain nAChR subtypes; for example, short-type α-neurotoxins almost as efficiently as the long-chain ones inhibit or stain (as radioactive or fluorescent derivatives) the muscle-type nAChRs but are considerably less efficient in respect to α7 or α9 nAChRs compared to such long-chain α-neurotoxins, such as α-bungarotoxin or α-cobratoxin. However, a certain disadvantage of the latter two is that they have a similar high affinity for the muscle-type α7 or α9 nAChRs, thus not allowing to independently prove the presence of a particular one.

A certain role in nAChR research is also played by a group of snake toxins, which were earlier considered to be weak toxins because they are of very low toxicity, and for most of them, the targets were not known. Structurally they are very close to the long-chain α-neurotoxins, but their additional 5th disulfide is not in central loop II, but it is in N-terminal loop I (Figure 1A). One such toxin, namely WTX (weak toxin), from *Naja kaouthia* snake venom, was shown to inhibit both muscle-type and α7 nAChRs at micromolar concentrations [10] and because of its low toxicity, may be considered for possible medical applications. Recent cryo-electron microscopy determination of the WTX structure in complex with the extracellular domain of the α7 nAChR [11] may open the way for the design of medically useful variants.

In connection with the WTX and other non-conventional toxins (such as candoxin) acting on the nAChRs [12], it is appropriate to consider other groups of three-finger proteins with the same disposition of disulfides, namely some proteins of the Ly6/uPAR family that also have a 5th disulfide in loop I. Many of them are known to be present in the immune system, but the similarity of some of them to the three-finger proteins from snake venom became obvious only after its discovery in the mammalian brain of protein Lynx1 (where Ly is from the Ly6 family and nx from neurotoxin) [13]. This protein is attached to the membrane by the glycosylphosphatidylinositol (GPI) anchor and was shown to affect the functioning of several subtypes of nAChRs. At about the same time, a secreted form (having no GPI tail) named SLURP-1 (lymphocytic antigen-6/urokinase type plasminogen activator receptor-related peptide 1) was found in mammalian urine [14] and also shown to interact with nAChRs [15]. Information about these proteins can be found in several reviews [16–18], and here we will consider those recent publications devoted to the action of these proteins on the nAChRs, which more or less directly affected the functioning of the immune system or pain transmission.

It should be emphasized that the first effects on nAChRs were reported for overexpressed Lynx1 containing the GPI anchor, and only later wsLynx1 (water-soluble Lynx1), devoid of this tail, was prepared in *E. coli*. Its three-finger structure was confirmed by ^{1}H-NMR spectroscopy, and inhibition at 10 μM at the orthosteric sites of the *T. marmorata* nAChR and of the α7 nAChR via allosteric sites were demonstrated [19]. In the case of SLURP-1, the products obtained in different laboratories had various added fusion parts, and for them was reported either agonistic activity against α7 nAChR [15] or inhibition by an allosteric mechanism with SLURP-1, which differed from the naturally occurring product by only one additional N-terminal Met residue [20]. In one case, the product identical to the native SLURP-1 was prepared through peptide synthesis and shown to inhibit several neuronal nAChR subtypes, including α9α10 nAChR [21], which at present, is considered an appropriate target against neuropathic pain.

Endogenous SLURP-1 is believed to be an immunomodulatory protein [22]. It is known to be present in keratinocytes, and its mutations are associated with the skin disease Mal de Maleda, the reasons being mainly its interactions with α7 nAChR and some other nAChR subtypes. There is evidence that SLURP-1 facilitates the functional development of T-cells and increases ACh synthesis [23]. It was earlier shown that SLURP-1 decreased the production of TNFα by T-cells and downregulated IL-1 β and IL-6 secretion by macrophages [24]. SLURP-1 decreased the production of the inflammatory cytokines induced by TNFα [25]. It was recently detected on the mast cells, and the obtained results demonstrated that, in general, the activation of certain nAChR subtypes in the cholinergic

system plays a role in the regulation of stress-sensitive inflammatory responses but may have a surprising pro-inflammatory effect on healthy skin, driving IL1β expression if SLURP-1 is involved [26].

It should be emphasized that, at present, there are ^{1}H-NMR data for a number of those Ly6/uPAR proteins capable of interacting with nAChRs; in particular, it was shown that recombinant SLURP-1 contains two isomers and is very conformationally mobile [27].

The work on Lynx1 was performed along two lines: analyzing the effects of endogenous Lynx1 (containing the GPI anchor) or testing the effects of the added wsLynx1. Thus, experiments in mice demonstrated that the antinociceptive effects of nicotine and epibatidine were enhanced in the mice with the knockout (KO) of the Lynx1 gene. Experiments with selective antagonists revealed that here the main target should be the α4β2 nAChRs [28]. First, it was shown that wsLynx1 inhibited *T. californica* nAChR by binding at the orthosteric sites and inhibited α7 nAChRs by attaching at the allosteric sites [20], the IC$_{50}$ values in both cases being around 50 μM. However, recently, it was found that at 2 μM, wsLynx1 increased the ACh-induced currents in rat neurons, which might be used for designing drugs against neurodegenerative diseases [29]. In the same publication, the authors provided some indications that endogenous Lynx1 has an opposite action on the activation of α7 nAChRs, which is in accord with the results produced by other laboratories. It should be noted that some of the inhibiting activities of wsLynx1 and of the other proteins of the Ly6/uPAR family expressed in *E.coli* can be reproduced by their synthetic fragments with stabilized spatial structures, which can open new pathways to drugs; in particular, the fragment of the central loop of wsLynx1 had the same capacity to inhibit the muscle-type nAChR of *T. californica* as wsLynx1 [30,31]. In conclusion, it should be mentioned that there are almost no publications where the interaction of the Ly6 proteins with the nicotinic receptors would be shown to affect the pain signal transmission, but because recent data demonstrate the activity of SLURP-1, wsLynx1 and some other water-soluble forms of the Ly6 proteins against different cancer cells [32–35] it might be expected that such proteins produce some analgesic effects as well.

4. α-Conotoxins in Distinguishing the Individual nAChR Subtypes

α-Conotoxins, short neurotoxic peptides found in venoms of *Conus* mollusks targeting different nAChR subtypes, are the most accurate tools, allowing not only for distinguishing muscle-type nAChRs from neuronal ones but also the most precise identification of individual subtypes of neuronal nAChRs. There are many recent reviews covering this field (see, for example, [36–38]); thus, here we will briefly present information relevant to the α7 and α9α10 nAChRs, which are the focus of our review. Contrary to the complexes with the snake venom three-finger protein neurotoxins, there are not yet X-ray or cryoEM structures of α-conotoxins bound to whole-size nAChRs; the information related to the binding sites is based mainly on the X-ray structures of α-conotoxin complexes with AChBPs (see, for example, reviews [39,40], Figure 1D).

The first discovered α-conotoxin acting on neuronal nAChRs was α-conotoxin ImI. However, it showed multiple specificities, interacting not only with the α7 and α9 subtypes [41] but also with different heteromeric neuronal nAChRs [42,43]. In subsequent years, a number of new peptides interacting with α7 nAChR, namely α-conotoxins ImII [44], EpI [45], PnIB [46], AnIB [47], GID [48], OmIA [49], Vc1.2 [50], RegIIA [51], LsIA [52], CIB [53], G1.5 [54], MrIC [55], Lo1a [56], BnIA [57] and AusIA [58] were purified from venoms or derived from mRNAs isolated from the poisonous ducts of various mollusks. However, all of them either also showed multiple specificities or, similar to the last four, had a low affinity for the α7 receptor. α-Conotoxins ArIA and ArIB proved to be the most potent (although also non-selective) ligands with nanomolar affinity for α7 nAChR [59]. The situation with selectivity was partially solved by obtaining a large number of analogs of naturally occurring α-conotoxins. Thus, more potent and/or selective (towards α7 nAChR) analogs of α-conotoxins ImI [60] and PnIA [46,61,62], as well as species-selective (human/rat) peptides based on α-conotoxins RegIIA, TxIB [63] and LvIB [64] were de-

signed and synthesized. Some of the most successful in this direction were analogs of α-conotoxin ArIB. The double mutation [V11L, V16A] increased potency (IC$_{50}$ 0.36 nM) and another double-mutated [V11L, V16D] analog became the most specific and sufficiently active (IC$_{50}$ 1.1 nM) ligand for homomeric α7 nAChR [59,65]. On the basis of the first of them, radioactive and fluorescent forms were prepared, which were successfully used for detecting α7 nAChR on various cellular preparations [66,67]. Currently, α-conotoxin ImI, analogs of PnIA and ArIB, are most often used to study the role of α7 nAChR in different cells and in various physiological processes, including inflammatory ones [68–70].

The list of conotoxins targeting α9α10 nAChR is also being updated, starting with the above-mentioned non-selective α-conotoxin ImI. In the 2000s, the α-conotoxins Vc1.1 [71,72], PeIA [73] and RgIA [74], most commonly used in studies on α9α10 receptors, were discovered. The ability to interact with this nAChR subtype was also revealed for the later-discovered α-conotoxins Vc1.2 [50], Mr1.7 [75], GIB, G1.5 [54] and Mr1.1 [76]. However, with the exception of RgIA, all other peptides showed multiple selectivities. For α-conotoxin RgIA, the X-ray structure has been established in a complex with the extracellular ligand-binding domain of the α9 subunit of human nAChR [77] (Figure 1E). It revealed the important residues of conotoxin, but it should be kept in mind that this domain is monomeric and has only one binding surface.

The particular interest in peptides targeting α9α10 nAChR was due to their revealed ability to inhibit neuropathic pain in in vivo rodent models. This was shown primarily for α-conotoxins Vc1.1 and RgIA [72,78]. However, the involvement in this analgesic effect of α9α10 nAChR was disputed [79], pointing to the inhibition of N-type calcium channels via GABA-B receptor activation [80,81]. In addition, it was revealed that the affinity of α-conotoxins Vc1.1 and RgIA to human α9α10 nAChR is significantly lower than that of rats [82,83]. All this led to the design of analogs that are highly selective toward human α9α10 nAChR. In particular, several potent and selective analogs of α-conotoxins Vc1.1 [84] and RgIA [85–87] were synthesized. One of the analogs of RgIA, namely RgIA4, prevented chemotherapy-induced neuropathic pain mediated through action on α9α10 nAChR; therefore, it is now considered a possible analgesic [88].

It is worth mentioning that the ability to interact with α9α10 nAChR was also shown by representatives of another group of conotoxins, namely the αO-conotoxins GeXIVA [89] and GeXXVIIA [90], αS-conotoxin GVIIIB [91] and αD-conotoxin Lt28.1 [92]. The most promising among them is αO-conotoxin GeXIVA, which exhibits high selectivity when targeting this receptor subtype with nanomolar affinity. In addition, in experiments on rodents, analgesic effects were demonstrated, mediated by the interaction with α9α10 nAChR [93,94]. This peptide contains 22 amino acid residues, being unusually long compared to typical α-conotoxins. Moreover, contrary to α-conotoxins acting on the α9α10 nAChRs via the receptor orthosteric binding sites, αO-conotoxin GeXIVA inhibits its target by attaching to some allosteric sites [89]. Another untypical feature of this conotoxin is the large number (9) of arginine residues. This was the reason for the synthesis of oligoarginine peptides of different lengths and testing their activity against nAChRs. It was found that, depending on the peptide length, oligoarginines with different efficiencies inhibited various nAChR subtypes. The highest activity (in the nanomolar range) was observed for the hexadecaarginine peptide R16 and octaarginine R8, the latter being the most efficient against the α9α10 nAChR [95]. Recently, the action of a series of oligohistidines, oligolysines and oligoarginines was tested against nAChRs, oligoarginine R9 having a higher affinity than R8 toward α9α10 nAChRs [96]. Here, it should be noted that oligoarginines were known mainly as a means for the intracellular delivery of various compounds attached to them. There are also data showing that oligoarginines can bind to the NMDA receptors and serve as potential means against neurodegenerative diseases [97], but their binding to the nAChRs was demonstrated in [95] for the first time. A comparison of the effects of R8 and α-conotoxin RgIA against neuropathic pain in a mouse model will be considered later in this review.

5. Functional Roles of nAChR in Immune Cells

5.1. Non-Neuronal Cholinergic Anti-Inflammatory Reflex

Vagus nerve signaling is an important part of the afferent loop that modulates responses to systemic endotoxemia. Efferent vagal signaling can promote the release of lymphocytes from the thymus via nAChRs [98]. In addition, the activation of the vagus nerve by endotoxin or cytokines stimulates anti-inflammatory responses. ACh is the main mediator of the vagus nerve. It has previously been found that exposure to ACh on lipopolysaccharide-activated human macrophages results in the significant inhibition of the release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin (IL)-1b, IL-6 and IL-18 but not of the anti-inflammatory cytokine IL-10 [99]. These results showed that the vagus nerve, previously thought to be activated only in response to peripheral inflammation, is also capable of modulating inflammatory responses. The mechanisms underlying the interaction between the nervous and immune systems were later called the cholinergic anti-inflammatory pathway (CAP) [100]. This pathway plays a significant role in controlling the inflammatory process by interacting with the nAChRs expressed on macrophages.

Vagal modulation of immune responses can occur in the densely innervated gastrointestinal tract. The parasympathetic nervous system can also control immune responses to commensal flora and food components [101]. The dietary intake of fats stimulates the production of cholecystokinin (CCK), which is a neuropeptide essential for triggering several digestive functions, including exocrine pancreatic secretion and the activation of afferent vagus nerve signals. Studies have shown that CCK released from high-fat enteral nutrition inhibited the hemorrhagic-shock-induced release of TNF-α and IL-6 [102]. This anti-inflammatory effect of CCK is mediated by the vagus nerve, as surgical or chemical vagotomy abolished the anti-inflammatory effect of both the high-fat diet and CCK administration. Accordingly, the activation of the vagus nerve prevents surgically induced inflammation of the intestinal muscles and improves postoperative ileus [103].

The identification of major macrophage-expressed receptors involved in the CAP was initially a major challenge. Macrophages are the main cells producing TNF-α [104]. Studies using genetic knockout technology and α7 nAChR-deficient mice showed that TNF-α levels in such mice were significantly higher after endotoxin administration than in wild-type mice. Similarly, electrical stimulation of the vagus nerve in α7-deficient mice did not lead to a decrease in serum TNF-α levels during endotoxemia, while in wild-type mice, a significant decrease in the level of TNF-α was observed [105]. Thus, it was concluded that the inhibition of TNF-α mediated by the vagus nerve in vivo depends on α7 nAChRs.

Further animal studies have shown that splenic nerve stimulation also inhibits TNF-α production [106]. The α7 nAChR agonist choline attenuated serum TNF-α levels in mock mice but not after splenic neurectomy [107,108]. These results suggest that α7 nAChR is a postsynaptic receptor required for splenic nerve activation and for controlling inflammatory responses. The research results show that the vagus nerve interacts functionally with the splenic nerve [108]. Electrical stimulation of the rat splenic nerve, as well as the activation of macrophage β-adrenergic receptors by norepinephrine, reduced LPS-induced TNF-α release. In this case, ACh released by the vagus nerve leads to the activation of the α7 nAChRs expressed in the ganglia of the mesenteric plexus and modulates the function of the splenic nerve through the release of norepinephrine in the spleen. The elucidation of the molecular mechanism involved in the splenic nerve in the CAP showed that that splenic nerve endings positive for β2-adrenergic receptor form synapse-like structures on T lymphocytes containing choline acetyltransferase (ChAT$^+$) and synthesizing ACh – agonist of α7-receptors [109,110]. Thus, ChAT$^+$ T-lymphocytes were an important intermediate step between the splenic and vagus nerve, which was necessary for the inhibition of the endotoxin-induced release of TNF-α via α7 nAChR expressing macrophages [98].

It is becoming clear that ACh is an important component of CAP, which regulates various immune processes. It has been established that various types of immune cells, such as macrophages, dendritic cells and T- and B-cells, produce ACh, which, by activating α7

nAChR, triggers an anti-inflammatory immune response [111,112]. Immune cells possess all the components of an independent cholinergic system, suggesting that ACh synthesized by immune cells plays a key role in the regulation of immune function [113]. Neuroimmune interactions mediate various functional and biochemical effects through AChRs expressed on immune cells. The importance of various components of CAP in immune cells is discussed below.

5.2. T-Cells

Fujii et al. [114] showed that the activation of the human T-cell lines Molt-3, CEM and HSB-2 by phytohemagglutinin (PHA) leads to the accumulation and release of ACh. The results obtained suggest that the ACh released by T-cells interacts with cholinergic receptors, thereby leading to the modulation of the immune system. In mononuclear human lymphocytes, PHA stimulation resulted in the induction of ChAT mRNA, leading to the synthesis of ACh [115]. The expression of $\alpha9$ and $\alpha10$ subunits has been demonstrated in T- and B-cell line but no ion currents have been reported in response to ACh [116].

Further studies identified that TCR/CD3 (T-cell receptor)-mediated T-cell activation increased ChAT expression and ACh synthesis [117]. The study of the role of the cholinergic system in Crohn's disease showed the presence of ChAT$^+$ T-cells in the spleen and Peyer's patches of the small intestine, which are capable of synthesizing ACh and contribute to the alleviation of colitis [118]. Activated spleen cells from $\alpha7$ receptor knockout mice produced higher levels of TNF-α, interferon (IFN)-γ and IL-6 [119]. The presence of functional nAChRs, including the $\alpha7$ type, in human T-cells, has been confirmed. The activation of nAChR in human T-cells with nicotine led to an increase in FasL expression and the transition of cells to the G0/G1 phase [120]. Treating dendritic cells with nicotine resulted in their low ability to stimulate antigen-presenting cell-dependent T-cell responses [121].

A study of the role of $\alpha7$ nAChRs expressed on T-cells and antigen-presenting cells (APCs) caused by GTS-21, a selective partial agonist of $\alpha7$ nAChR, showed that the stimulation of $\alpha7$ receptors suppresses the development of CD4$^+$ T-cells, reduces the ability to present antigens, and also enhances the differentiation and proliferation of both regulatory T-cells (Tregs) and effector T-cells via the activation of JAK2/STAT signaling pathways [122]. Another study also demonstrated that nicotine reduced T-cell proliferation as well as Th1 cytokine production and facilitated the transition to Th2 but had no effect on T-cells derived from $\alpha7^-/^-$ mice [123]. The immunosuppressive function of CD4$^+$CD25$^+$ Treg lymphocytes mediated by the activation of the $\alpha7$ receptor has been established. The stimulation of mouse Treg lymphocytes with nicotine increased the expression of cytotoxic T-lymphocyte-associated antigen (CTLA)-4 and transcription factor p3 (Foxp3). At the same time, these effects were stopped by the selective $\alpha7$ nAChR antagonist, α-bungarotoxin [124]. The treatment of mouse Treg cells with nicotine resulted in the suppression of TGF-$\beta1$ production but did not affect cell proliferation and IL-10 release, being mediated by $\alpha9$ nAChR [125].

Recent studies have shown that the activation of $\alpha7$ nAChR on CD4$^+$ lymphocytes by GTS-21 may promote the transcription of HIV-1 proviral DNA. The activation of the $\alpha7$ receptor increased the production of reactive oxygen species, decreased DUSP1 and DUSP6 and increased p38 MAPK phosphorylation [126].

5.3. B Cells

B-cells express ChAT and produce ACh, influencing the functions of the innate immune response [127]. Various subtypes of nAChRs are expressed in murine B lymphocytes, including $\alpha4$, $\alpha5$, $\alpha7$, $\beta2$ and $\beta4$ nAChR subunits. The expression of $\alpha4$, $\alpha7$ and $\beta2$ subunits was found to be significant for the maturation of B-cells [128]. Knockout mice lacking $\beta2$ or $\alpha7$ subunits had a reduced number of B-lymphocytes, and their treatment with nicotine resulted in a marked increase in the number of B-cells in the bone marrow [129]. The deficiency of $\alpha4$, $\alpha7$ and $\beta2$ containing nAChRs in B lymphocytes affected the rate of switching from IgM production to IgG production. The CD40-stimulated activation of B-lymphocytes derived from $\beta2^-/^-$ mice was more sensitive to anti-CD40, while nicotine attenuated the

anti-CD40 response [130]. The proliferation of unstimulated B lymphocytes in the presence of the α7 nAChR antagonist methyllycaconitine (MLA) was enhanced, with simultaneous inhibition of LPS-induced IgM production. B-cells from $\alpha7^-/^-$ mice produced fewer Foxp3$^+$ cells, and their induction in wild-type mice, as well as IL-10 production, was inhibited by α7 nAChR ligands [131]. Taken together, these studies demonstrate that nAChRs regulate mouse B-lymphocyte proliferation and Ig production, pointing to the importance of the cholinergic regulation of the humoral immune response and immunosuppression.

5.4. Dendritic Cells (DC)

Dendritic cells are APCs of the immune system, causing the activation of T-cells and differentiating them into functionally different Th1- and Th2-type cells [132]. The effect of nicotine on DC resulted in the reduced production of IL-1β, IL-10 and TNF-α, especially IL-12. DC-mediated T-cell proliferation and IFN-γ release were reduced. Nicotine had a direct effect on the ability of DC to polarize Th1 cells, indicating reduced immunostimulatory functions of DC [121,133]. CD205$^+$ DC secrete SLURP-1. Recombinant SLURP-1 attenuated the proliferation of peripheral blood mononuclear cells and increased the amount of ACh in the Molt-3 cells. The selective α7 nAChR antagonist MLA abolished these effects, suggesting an α7-receptor-dependent modulation of immune responses by SLURP-1 [134]. The α7 nAChR agonist GTS-21 improved the clinical course of arthritis in a mouse model of collagen-induced arthritis (CIA) by reducing the secretion of pro-inflammatory cytokines and downregulating CD80 and MHC II expression on the DCs of mice [135]. GTS-21 suppressed the APC-dependent differentiation of CD4$^+$ T-cells in Tregs. Ovalbumin-induced release of IFN-γ, IL-4 and IL-17 from the spleen cells of TCR transgenic mice was reduced by GTS-21. However, antagonists of α7 nAChRs (α-bungarotoxin and MLA) did not abolish the effects of GTS-21, which may indicate a pharmacological difference between non-neuronal α7 nAChRs expressed on DCs and those in neurons [136]. DC polarization under the influence of ACh led to the stimulation of OX40L expression, the induction of the Th2 profile and increased production of IL-4, IL-5 and IL-13 by CD4$^+$ T-cells [137]. Nicotine exposure to mouse and human DCs resulted in the increased expression of CD86 relative to CD80 and in the production of less IL-12, modulating the Th1/Th2 balance toward Th2 [138]. In a mouse model of sepsis-induced acute lung injury (ALI), GTS-21 reduced DC maturation and the production of pro-inflammatory cytokines, thereby reducing inflammatory responses in ALI [139].

5.5. Monocytes

Monocytes play an important role in the primary innate immune response. The human monocytic cell line U937 expresses various subtypes of nAChRs. In LPS-stimulated U937 cells, the nAChR agonist epibatidine inhibited the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-18) [140]. The treatment of monocyte THP-1 upon LPS activation with nicotine or the specific α7 nAChR agonist GSK1345038 resulted in the inhibition of TNF production [141]. The exposure of the primary human monocytes to nicotine resulted in the inhibition of the IL-18-enhanced expression of ICAM-1, B7.2 and CD40 and in the production of IL-12, IFN-γ and TNF-α by lymphocytes. The nonselective nAChR antagonist mecamylamine and selective for α7 nAChR α-bungarotoxin abolished the effects of nicotine, suggesting its dependence on the α7 receptor [142]. Nicotine inhibited IL-18 and IL-12 production in LPS-stimulated monocytes [143]. The activation of α7 nAChR in mouse Ly6C^{hi} spleen monocytes by GTS-21 suppressed TNF-α production. Exposure to various TLR ligands in the GTS-21-primed J774 monocytes resulted in the mRNA inhibition of TNF-α and IL-1β expression, increased histone deacetylation and inhibited NF-kB p65. These results indicate that α7 nAChR activation confers "anti-pro-inflammatory" memory to monocytes [144]. In mouse bone marrow cells, the α7 and α9 nAChR subtypes are the most common. Nicotine reduces the total number of monocytes and inhibits the IFN-γ-induced increase in pro-inflammatory monocytes. Nicotine exposure resulted in the inhibition of the production of pro-inflammatory cytokines TNF-α, IL-1β

and IL-12 by monocytes in the bone marrow while simultaneously stimulating the secretion of IL-10 [145]. Nicotine, acting on the α7 and α9 receptor subtypes, significantly inhibits the infiltration of pro-inflammatory monocytes and neutrophils into the CNS in a model of experimental autoimmune encephalomyelitis (EAE). mRNA levels for the chemokines CCL2 and CXCL2 are downregulated in the brains of nicotine-treated EAE mice prior to the massive infiltration of these cells, suggesting the importance of nAChRs in the treatment of neuroinflammatory diseases [146]. The administration of GTS-21 agonist provides effective elimination of pathogenic microorganisms, reduced inflammatory response and organ damage in the model of polymicrobial septic peritonitis. Stimulation by GTS-21 leads to the enhanced recruitment of monocytes into the abdominal cavity and to a simultaneous increase in phagocytic activity and the iNOS expression of these recruited monocytes [147].

A novel cholinergic mechanism has been discovered that inhibits the ATP-dependent release of IL-1β by human monocytes via nAChRs. It has been shown that phosphocholine (PC) is an agonist for monocytic nAChRs containing α9 and α10 subunits. PC and choline (Cho) inhibited the ATP-induced release of IL-1β from LPS-primed human monocytic U937 cells with the participation of α9 nAChR, which was confirmed by using conotoxin RgIA4, a selective antagonist of this receptor subtype [148]. At the same time, a metabotropic mechanism of action of PC on monocytic nAChR was assumed. ACh and nicotine (Nic) also completely inhibited the release of IL-1β from U937 cells primed with LPS and stimulated with BzATP. α-Conotoxin ArIB[V11L, V16D], a selective α7 nAChR antagonist, abolished the inhibitory effects of ACh and Nic [149]. Subsequently, a number of compounds were identified which, by interacting with α7 and α9 receptors, lead to the inhibition of IL-1β release: a synthetic surfactant, palmitoylphosphatidylglycerol and dipalmitoylphosphatidylcholine (DPPC), lysophosphatidylcholine (LPC) and glycerophosphocholine (G-PC), PC-modified lipooligosaccharides (PC-LOS) from *Haemophilus influenzae* and C-reactive protein [69,150–153].

5.6. Macrophages

The mRNA expression of the α9 and α10 nAChR subunits was found in murine alveolar macrophages. However, $[Ca^{2+}]i$ in response to nicotine and ACh was not detected [154]. The stimulation of human macrophages (MDMs), as well as the murine macrophage RAW 264.7 cell line, with cholinergic agonists, resulted in a significant decrease in TNF production. The activation of α7 nAChR induces CREB-dependent transcriptional changes in macrophages, resulting in reduced TNF production in response to endotoxins [155]. ACh attenuated the release of various pro-inflammatory cytokines in LPS-activated MDMs, such as TNF, IL-1β, IL-6 and IL-18, but not of anti-inflammatory IL-10 [99]. The functional expression of α7 nAChRs on MDMs and macrophages from the monocytic THP-1 cells has been confirmed using a selective agonist PNU 282981. The activation of α7 receptors on macrophages led to an increase in the expression of membrane proteins HLA-DR, CD11b and CD54 but to a decrease in the expression of CD14 and in the production of IL-10 [156]. The anti-inflammatory effects of nicotine in peritoneal macrophages have been associated with the activation of the Jak2/STAT3 signaling pathway [103]. In LPS-activated macrophages RAW 264.7, agonists such as ACh and PNU 282987 inhibited MMP-9 (matrix metalloproteinase) production and cell migration; ACh activated the expression of JAK2 and STAT3. At the same time, the α7-receptor antagonist MLA abolished all the effects caused by cholinergic agents in cells [157]. The activation of the α7-receptor in LPS-stimulated RAW 264.7 macrophages resulted in the inhibition of mRNA expression and the production of TNF-α, IL-6, and IL-1β. α7 nAChR antagonists, such as MLA, α-cobratoxin and α-conotoxin PnIA[A10L], showed the opposite effect and increased the production of pro-inflammatory cytokines [70].

Using a mouse model of intracerebral hemorrhage, it was found that PNU 282987 leads to a decrease in inflammatory factors and contributes to the polarization of macrophages into an anti-inflammatory phenotype. The activation of the α7-receptor promoted au-

tophagy by increasing LC3 protein (Beclin) and the recovery of brain and heart function [158].

Specific α7-receptor agonists reduced the levels of the pro-inflammatory cytokines MIP-2 and TNF-α and the activation of nuclear factor NF-κB in a rodent model of acute lung injury, which was mediated by the activation of α7-receptors expressed by alveolar macrophages [159]. The administration of GTS-21 to mice with LPS-induced inflammation inhibited the production of TNF-α but not IL-6, by alveolar macrophages [160].

Chronic inflammation is also observed in viral infections, such as that of HIV. The CCR5 and CXCR4 glycoproteins of HIV tropic variants, such as gp120$_{JRFL}$ and gp120$_{IIIB}$, lead to the activation of α7 receptors on MDMs. A paradoxical pro-inflammatory phenotype was observed in macrophages, resulting in a failure to inhibit the release of pro-inflammatory cytokines, thus indicating the disruption of CAP in MDMs [161,162].

5.7. Mast Cells

The rat mast/basophil cell line RBL-2H3 expresses α7, α9 and α10 nAChRs. Nicotine exposure resulted in the inhibition of C4 leukotriene, TNF-α and IL-1β production after cell activation with FcεRI but had no effect on histamine release. These effects were associated with the inhibition of cytosolic phospholipase A$_2$ activity and the PI3K/ERK/NF-κB pathway and were blocked by α7/α9-nAChR antagonists [163]. The treatment of rat mast cells with low concentrations of ACh caused the release of histamine [164]. The binding of fluorescently labeled α-bungarotoxin to human mast cells HMC-1 indicated the presence of nAChR expression [165]. Human mast cells contain high levels of acetylcholinesterase. Cholinergic agonists cause mast cell degranulation but do not affect leukotriene B or TNF-α secretion [166]. The treatment of mouse bone marrow mast cells with α7-receptor agonists significantly inhibited antigen-induced degranulation, indicating the downregulation of mast cell activation via α7 nAChR [167].

5.8. Neutrophils and Granulocytes

Mouse polymorphonuclear neutrophils express nAChRs. Nicotine and ACh modified the respiratory burst and affected neutrophil adhesion, indicating a regulatory role of α7, α3β2, or α6 receptor types in neutrophil function [168]. Nicotine, choline and other cholinergic agents induced Ca^{2+} transients in polymorphonuclear neutrophilic granulocytes, increasing cell adhesion and decreasing the production of reactive oxygen species, which was mediated by α9 nAChR [169]. The treatment of neutrophils with nicotine led to the release of neutrophil extracellular traps and the activation of enzymes Akt and PAD4 [170]. In a model of sepsis-induced acute lung injury, the administration of α7 nAChR agonists resulted in a decrease in the transalveolar migration of neutrophils [171].

5.9. Natural Killer (NK) Cells

Natural killer (NK) cells are effector lymphocytes that control several types of tumors and microbial infections [172]. Human NK cells express α7 nAChR. In cytokine-stimulated NK cells, the activation of α7 receptors by PNU 282987 suppressed NKG2D expression but did not affect NKp46 (CD335; cytotoxicity-activating receptor) and DNAM-1 (CD226; accessory molecule). NK cells exposed to a specific α7 nAChR agonist exhibited reduced cytotoxic activity and IFN-γ production and showed reduced p65 NF-κB nuclear mobilization. The co-cultivation of PNU 282987-treated NK cells with DC resulted in significantly lower MHC-II and CD83 expression in DCs and a lower percentage of CD86^{high} DCs [173]. Mouse NK cells express ChAT and synthesize higher amounts of ACh during inflammation. ChAT$^+$ NK cells and CCR2$^+$Ly6C^{hi} monocytes form immune synapses, which helps to reduce infiltration and the production of pro-inflammatory cytokines [174]. Another study reports that nicotine impairs the ability of NK cells to kill cancer cells and release cytokines. The treatment of mouse NK cells with nicotine resulted in the decreased expression of NKG2D (CD314; type II integral membrane protein), Ly49I (homodimer type II transmembrane protein) and diminished cell proliferation. NK cells exposed to nicotine were less

effective at killing B16 melanoma tumor cells [175]. Rat spleen NK cells treated with ACh also showed reduced lysis of Yac-1 lymphoma cells [176].

To conclude this section, it can be said that ACh is produced by various types of immune cells and is involved in many biological processes. Agonists of nAChRs, such as ACh and nicotine, can act on different types of immune cells through autocrine or paracrine stimulation. The data related to the biological effects of the actions on the α7, α9 and α9α10 nAChRs in different immune cells are summarized in Table 1. The cholinergic anti-inflammatory pathway is an important mechanism activation of which, by nAChRs, leads to the inhibition of cytokine production. Vagus nerve stimulation of ACh release, as well as the administration of cholinergic agonists, reduce cytokine production in various models of inflammation. The activation or inhibition of nAChRs opens up new therapeutic possibilities for the effective treatment of various inflammatory, autoimmune and infectious diseases.

Table 1. The biological effects of the actions on the α7, α9 and α9α10 nAChRs in different immune cells.

Immune Cell Type	nAChR Subtype	Agonists and Antagonists	Biological Effect	References
T-cells	α7	Nicotine	Increased FasL expression and suppressed the development of CD4+ T-cells	[120]
	α7	GTS-21	Enhanced the differentiation and proliferation of Tregs and effector T-cells	[122]
	α7	Nicotine	Reduced T-cell proliferation and Th1 cytokine production	[123]
	α7	Nicotine	Increased expression of CTLA-4 and Foxp3	[124]
	α9	Nicotine	Suppressed TGF-β1	[125]
	α7	GTS-21	Promoted transcription of HIV-1 proviral DNA, increased reactive oxygen species, decreased DUSP1 and DUSP6; increased p38 MAPK phosphorylation	[126]
B cells	α7	MLA	Enhanced proliferation	[131]
Dendritic cells	α7	Nicotine	Reduced production of IL-1β, IL-10, TNF-α and IL-12	[121]
	α7	SLURP-1	Attenuated cell proliferation	[134]
	α7	GTS-21	Reduced secretion of pro-inflammatory cytokines and downregulation of the CD80 and MHC II expression	[135]
	α7	GTS-21	Suppressed APC-dependent differentiation of CD4+ T-cells	[136]
	n.d. *	Acetylcholine	Stimulated OX40L expression, induced Th2 profile, increased production of IL-4, IL-5, and IL-13 by CD4+ T-cells	[137]
	n.d.	Nicotine	Increased expression of CD86 and production of less IL-12, modulation of the Th1/Th2 balance towards Th2	[138]
Monocytes	α9α10	Epibatidine	Inhibition of pro-inflammatory cytokines	[140]
	α7	Nicotine and GSK1345038	Inhibition of TNF production	[141]
	α7	Nicotine	Inhibition of IL-18-enhanced expression of ICAM-1, B7.2 and CD40 and production of IL-12, IFN-γ and TNF-α	[142]
	α7	GTS-21	Suppressed TNF-α production	[144]
	n.d.	Nicotine	Inhibition of the production of TNF-α, IL-1β and IL-12 and stimulation of the IL-10 secretion	[145]
	α9α10 and α7	Nicotine, acetylcholine, phosphocholine	Inhibition of ATP-induced release of IL-1β	[148,149]

Table 1. *Cont.*

Immune Cell Type	nAChR Subtype	Agonists and Antagonists	Biological Effect	References
Macrophages	n.d.	Acetylcholine	Attenuation of the release of TNF, IL-1β, IL-6 and IL-18, but not IL-10	[99]
	α7	PNU 282987	Increased expression of HLA-DR, CD11b and CD54; decreased expression of CD14 and of IL-10 production	[156]
	α7	Acetylcholine and PNU 282987	Inhibition of MMP-9 production and cell migration	[157]
		GTS-21	Inhibition of TNF-α production	[160]
Neutrophils and granulocytes	α7 and α9	Nicotine and acetylcholine	Modified respiratory burst and affected neutrophil adhesion	[168]
	α9	Nicotine and choline	Increased cell adhesion and decreased reactive oxygen species production	[169]
	n.d.	Nicotine	Release of NET, activation of Akt and PAD4	[170]
Mast cells	α7 and α9	Nicotine	Inhibition of C4 leukotriene (LTC4), TNF-α, and IL-1β	[163]
		Acetylcholine	Induced release of histamine	[164]
Natural killer cells	α7	PNU 282987	Suppressed NKG2D expression, reduced cytotoxic activity and IFN-γ production	[173]
	n.d.	Nicotine	Impairment of the ability of NK cells to kill cancer cells and release cytokines; decreased the expression of NKG2D, Ly49I and cell proliferation	[175,176]

* nAChR subtype was not determined.

6. α7- and α9-Containing nAChRs in Chronic Pain

6.1. α7 nAChR in Pain

The analgesic properties of drugs selective to α7 nAChR have attracted significant attention after the discovery of the leading role of this receptor subtype in the vagal-mediated CAP (see part 5.1 of this review). The expression of α7 nAChR along pain transmission pathways has been known for a long time (reviewed in [177]). Moreover, the protein level of α7 nAChR is significantly downregulated in the sciatic nerve, dorsal root ganglion (DRG) and spinal cord in animal pain models of different etiology, providing a cellular and molecular basis for the known alleviation of chronic pain, including neuropathic pain, inflammatory pain and cancer-induced bone pain owing to activation or positive modulation of α7 nAChR (reviewed in [178]). Consistent with this was the observed decrease in the expression of α7 and β2 nAChR in the spinal cord and midbrain periaqueductal gray of hyperalgesic rats, which was reversed by analgesic electroacupuncture stimulation [179]. Potentially, not only homopentameric α7 but also heteromeric α7β2 nAChRs might be involved in pain regulation. There are some excellent recent reviews about the role of α7 nAChR and its specific ligands in pain modulation [177,178,180]. In this article, we are going to briefly review the newest tendencies and studies published in this field in the last two years.

Potent antinociceptive effects have been shown for α7 nAChR-targeting full, partial and silent agonists and positive allosteric modulators (reviewed in [177,178,180]). However, there is a tendency toward the reconsideration of the subtype-specificity of compounds acting on nAChRs. For example, among α7-selective partial and silent agonists, including those possessing anti-inflammatory and antinociceptive activity [181], some new α9-specific potent agonists and antagonists have been discovered [182]. The results of work with phosphocholine and pCF3-diEPP, silent/partial agonists of both α7 and α9-containing nAChRs, suggest that the responses to agonists by both receptor subtypes may be the basis for the observed anti-inflammatory effects in monocytic and macrophage-like cells [183].

CHRFAM7A is a relatively recent and exclusively human gene arising from the partial duplication of exons 5 to 10 of the α7 nAChR-subunit-encoding gene, CHRNA7 [184]. CHRFAM7A translates the dupα7 protein in a multitude of cell lines and heterologous systems while maintaining processing and trafficking that are very similar to those of the full-length form. Two isoforms of the CHRFAM7A gene transcripts code dupα7 proteins, which are short of a part or of the entire binding site but contain all of the α7 transmembrane domain sequences [185]. The co-expression of this gene with full-length α7 has dominant negative effects on ion channel function [186–188] and on the α7-nAChR-mediated control of exocytotic neurotransmitter release [189]. Through the regulation of the cholinergic anti-inflammatory pathway (reviewed in [177,190]), the CHRFAM7A gene expression level is linked to the severity of inflammatory-related pathologies, such as sepsis, osteoarthritis [191,192], cerebral ischemia/reperfusion injury, hypertrophic scars, COVID-19, inflammatory bowel disease [193], renal fibrosis [194] and pain, including neuropathic pain after spinal cord injury [195] and inflammatory pain due to osteoarthritis modeling [192].

6.2. α9-Containing nAChR in Pain

The first indications of the analgesic effects mediated by α9α10 nAChR arose when this receptor subtype was identified as a molecular target for analgesic α-conotoxins Vc1.1 and RgIA, which alleviated neuropathic pain in rodent models (reviewed in [196–198]). In particular, α-conotoxin RgIA (daily i.m. injection of 2 or 10 nmol) and its analog RgIA4 (daily s.c. injection of 0.128–80 μg/kg dose), which is highly specific to both human and rodent α9α10 nAChRs, were analgesic in oxaliplatin-induced peripheral neuropathy in rats and mice [85,86,199]. The latter also accelerated recovery from paclitaxel-induced neuropathic pain [88]. Oxaliplatin and paclitaxel are first-line platinum- and taxane-based anti-cancer drugs, efficient against colorectal, breast, ovarian and non-small cell lung cancers [200,201]. The most common side effects of such chemotherapeutics are neurological reactions, namely chemotherapy-induced peripheral neuropathy (CIPN). Characteristic manifestations of CIPN are impaired cold sensitivity (cold allodynia) as well as headache, numbness, chronic pain and asthenia [202–204]. Currently, there is no entirely effective protocol to treat CIPN [205]. Our recent findings have shown that another α9α10 nAChR antagonist, oligoarginine R8, a member of a new class of nAChR inhibitors [95], was as effective as RgIA in alleviating oxaliplatin-induced neuropathic pain in mice at a five-times lower dosage (0.5 nmol (20 mg/kg) of R8 versus 2 nmol (100 mg/kg) of RgIA i.m. daily administration) [206]. Another α9α10-specific αO-conotoxin, GeXIVA, also alleviates chemotherapy-induced peripheral neuropathic pain induced by oxaliplatin [93] and paclitaxel [207] at a dosage of 0.45 mg/kg i.m. GeXIVA possesses four rather than six Cys residues, which is a unique structural property among members of the O1 conotoxin gene superfamily [89].

The blockade of α9α10 nAChR also provided positive effects in a rat chronic constriction injury (CCI) pain model, another model of neuropathy. Several specific α9α10 nAChR α-conotoxins Vc1.1, RgIA, Mr1.1 [S4Dap], αO-conotoxin GeXIVA [1,2] (≤2 nmol, i.m.) and small-molecule ZZ-204G (≥3.6 μg/kg) displayed analgesic activity in a CCI pain model [72,76,78,89,94,208,209]. Thus, α9α10 nAChR antagonists are considered to have great potential in relieving the neuropathic pain induced by diabetic peripheral neuropathy, the most common complication associated with long-term diabetes mellitus [210].

Repeated treatments with conotoxins RgIA, RgIA4, GeXIVA [1,2], oligoarginine R8 and small-molecule ZZ-204G inhibitors of α9α10 nAChR produced a cumulative analgesic effect without tolerance and, in some studies, promoted recovery from neuropathic pain [85,86,88,93,94,199,206,208,209]. A number of studies indicated that therapeutic doses of these substances, which were shown to be analgesic, did not impair rodent motor functions [89,93,206,209].

6.2.1. Molecular Mechanisms of Analgesia Mediated by α9-Containing nAChRs

The molecular mechanism of analgesia mediated by the inhibition of α9α10 nAChR is not fully understood yet. The analgesic effects of α9α10 nAChR inhibitors, reported for neuropathies of different origins (chronic constriction injury pain model and chemotherapy-induced neuropathy), may be partially realized by the direct antagonism of α9α10 receptors expressed in sensory DRG neurons. These neurons are pseudounipolar, with their axons outstretched to the spinal cord and periphery. DRG neurons express a number of nAChRs, including both the α9 and α10 nAChR subunits [211]. Some nAChRs have been shown to be axonally transported in DRG neurons and accumulated at the site of sciatic nerve ligation [212]. Such trauma provokes the development of neuropathic pain in CCI and PNL (partial nerve ligation) pain models. The possible involvement of peripheral neuronal α9α10 nAChRs in the analgesic effects of α-conotoxins Vc1.1, RgIA and αO-Conotoxin GeXIVA [1,2] is consistent with the observed acute analgesic effect of their intramuscular injection, developing within 1–4 h in CCI, PNL and chemotherapy-induced neuropathies in rat [72,78,93,94,207].

On the other hand, α-conotoxin treatment provokes multiple long-lasting processes. The analgesic effects of α-conotoxins Vc1.1 and RgIA administration continued for 7–12 days post-treatment in different neuropathy pain models [78,213]. The long-lasting analgesic effect of GeXIVA [1,2] on mechanical allodynia in CCI and oxaliplatin-treated rats continued for 10–14 days after the repeated intramuscular administration of the toxin was ceased [93,94]. The daily administration of RgIA4 (s.c., 40 μg/kg) reversed oxaliplatin-induced cold allodynia in mice, but only after 3 weeks of treatment [86]; in a rat paclitaxel-induced neuropathic pain model, its therapeutic effects reached significance 12 days after the last administration of RgIA4 (daily s.c. injection of 80 μg/kg for a month), which is suggestive of a rescue mechanism [88]. In addition, chronic α-conotoxins Vc1.1, RgIA and oligoarginine R8 administration led to neuroprotection, preventing CCI-induced degenerative changes both in the sciatic nerve structure [78,208] and oxaliplatin-induced DNA damage in dorsal root ganglion neurons in rat neuropathic pain models [199,206].

The observed long-lasting effects, including neuroprotection, might lead to the properties of α-conotoxins modulating the number and functioning of glial cells [199,208]. In CCI and oxaliplatin-treated rats, RgIA treatment prevented a numerical increase in microglia and astrocyte cell density present in the spinal cord, but it was able, per se, to elicit a numerical increase and morphological activation in microglia and astrocytes in specific brain areas, suggesting that RgIA may modulate glial cells in order to promote neurorestoration and reduce pain [199,208].

In addition, the role in pain relief through the actions on the α9α10 nAChRs present in the immune cells is becoming more evident. The blockade of α9α10 nAChR with α-conotoxins or small-molecule inhibitor ZZ-204G can alleviate both chronic neuropathic and inflammatory pain [209,214]. Potent α9-selective agonists, as well as numerous antagonists, have been described recently [182]. Several of these compounds have previously been shown to be effective in animal models of inflammatory pain, an activity that was assumed to be due to α7 silent or partial agonism but may, in fact, be due to α9 nAChR activity [181]. Moreover, the chronic constriction of the sciatic nerve produces an inflammatory response at the site of the injury [215]. This inflammatory response is thought to contribute to the development of neuropathic pain following peripheral nerve injury [215]. In rat CCI pain models, RgIA or Vc1.1 (0.2–10 nmol i.m.), in addition to their analgesic effects, significantly reduced edema and inflammatory infiltrate, including a decrease in macrophages and T-cells [72,208]. Recently, it has been shown that the RgIA4 prevention of acute oxaliplatin-induced cold allodynia requires both α9-containing nAChRs and CD3+ T-cells [216]. In this study, the subcutaneous administration of RgIA4 (40 μg/kg daily for 4 days) demonstrated an analgesic effect in wild-type mice, but not in mice lacking the α9 nAChR-encoding gene, *chrna9*, or depleted CD3+ T-cells. Thus, long-lasting α-conotoxin-induced analgesia may include specific activities of immune and glial cells and such α9α10 nAChR-mediated mechanisms remain an area of active investigation.

Several studies have proposed that α-conotoxins Vc1.1, RgIA and αO-conotoxin GeX-IVA can exert analgesic effects through the modulation of the N-type VGCC Ca$_V$2.2 via the stimulation of G protein-coupled γ-aminobutyric acid type B (GABA$_B$) receptors instead of the inhibition of α9-containing nAChR [217–224]. This is consistent with the observed inhibition of N-type VGCC CaV2.2 by α-conotoxins Vc1.1 and RgIA in DRG neurons of α9 KO mice [222]. Although such molecular mechanism of action is well-characterized for ω-conotoxin MVIIA (ziconotide), a recommended drug for first-choice intrathecal monotherapy against chronic pain [225], several studies have not fully reproduced such inhibitory effects on the high-voltage-activated calcium channels for the α- and αO-conotoxins [89,226–228]. Moreover, α-conotoxin RgIA4, a derivative of RgIA, lacks GABA$_B$ receptor activity but maintains the capacity of the parent peptide to prevent the development of neuropathic pain [85,86,88]. The studies carried out with the α9 subunit KO mice also prove the necessity of α9-containing nAChRs for chronic pain relief [85,216,227,229].

6.2.2. α-Conotoxin-Based Drug Development Strategies

Although α-conotoxins specific to α9α10 nAChR hold great pharmacological potential, they are not ideal therapeutic drug leads due to a number of shortcomings, such as a short half-life in vivo, unstable disulfide bonds, limited modes of administration and poor potency at human nAChRs for some of them [230–232]. In recent reviews [197,198], the established strategies for improving the activity, selectivity and stability of α-conotoxins have been considered in detail. They include scanning mutagenesis, unnatural amino acid substitutions, disulfide bond modification, backbone and side-chain cyclization and polymerization. Moreover, drug development for clinical use requires working out a stable medicinal formulation. In the case of conotoxins, lyophilization or encapsulation in microspheres could diminish their intrinsic instability and provide a sustained release of the drug to meet the clinical treatment needs of chronic pain [207,233]. In addition, new small-molecules [182,234] and peptide [235] inhibitors of α9-containing nAChRs are emerging, differing in chemical structure from known analogs. For example, a new A-superfamily conotoxin Bt14.12, similar to α-conotoxins and other A-superfamily conotoxins, contains a four Cys (C-C-C-C) framework, but with a unique disulfide bond connection "C1-C4, C2-C3". Interestingly, the addition of three Arg residues at the N-terminus of Bt14.12 enhances its inhibitory activity four-fold compared to wild-type Bt14.12, opening new perspectives of chimeric constructions between pain-relieving α9-specific conotoxins and oligoarginines [95,206]. Moreover, the discovery of several potent α9-specific agonists [182] suggested a new future direction for analgesic drug development.

7. α7- and α9-Containing nAChRs as Targets in Viral Infection

Nicotinic acetylcholine receptors are involved in inflammatory processes and nociception but are also direct targets recognized by a number of viruses. The first and canonical example here was a study on the interaction of the trimeric Rabies virus glycoprotein (RVG) with nAChRs, which began in the 1980s. At first, the homology of the central loop of snake α-neurotoxins with a certain fragment of the viral protein was revealed, and then the ability of this receptor-recognizing fragment 173–204 (as well as of the full-size RVG) to interact with the orthosteric binding site of the muscle and muscle-type nAChRs was demonstrated; polyclonal antibodies to this fragment were also obtained, which bound to cholinergic ligands. Since that time, attempts have been undertaken to use the RVG fragments of different lengths both to inhibit the binding of the virus and to facilitate the delivery of various compounds to the central nervous system. It is well known that the Rabies virus, after a period of replication in muscles, enters the central nervous system and selectively infects certain neuronal populations; therefore, the search for other targets of RVG continues. Among these targets were neural cell adhesion molecules (NCAMs), p75 neurotrophin receptor (p75NTR), metabotropic glutamate receptor subtype 2 (mGluR 2), integrin β1 and neuronal nAChRs. It should be noted that the function of neuronal

nAChR subtypes in Rabies virus infection is currently not well understood. However, the interaction was shown between full-length RVG ectodomain (as well as shorter RVG neurotoxin-like peptides) and α4β2 nAChR in vitro, which can explain the behavioral changes in *C. elegans* and mice [236]. The involvement of α7 nAChRs of the central and peripheral nervous systems in interaction with RVG is only assumed, but it has been shown that a recombinant trimeric RVG binds to α7 nAChRs expressed on monocyte-derived macrophages that induce the cholinergic anti-inflammatory pathway, characterized by a significant decrease in TNF α upon LPS challenge [237]. It has also been shown that RVG expressed in recombinant avirulent LaSota strain of Newcastle disease virus (rL-RVG) suppresses (as a competitive antagonist of α7 nAChR) the migration of gastric cancer cells by regulating α7 nAChR/ERK signaling and epithelial–mesenchymal transition [238]. A recently published cryo-EM structure of trimeric RVG complexed with RVA122, a potently neutralizing human antibody [239], should stimulate computer modeling of the RVG complexes with its targets to understand the molecular mechanism of the spread of the Rabies virus in an infected organism.

The first studies of the interaction of nAChRs with the human immunodeficiency virus (HIV), or rather, with its envelope glycoprotein, gp120, in many ways, resembled those seen for RVG. The reason for this was the identification of a certain homology of HIV gp120 fragment 159–169 with a fragment of Rabies virus glycoprotein and the central loop of snake α-neurotoxins recognizing distinct nAChR subtypes. This fact suggested a possible interaction of gp120 with cholinoreceptors, which was confirmed for the solubilized nAChR from fetal calf muscle [240,241]. However, the main target of action for HIV gp120 soon became α7 nAChR, represented both in the nervous system and on the immune cells (see, for example, the review [242]).

The role of this receptor in relation to HIV is currently being investigated in two main lines, namely, chronic inflammation (both neuroinflammation and peripheral inflammation) and HIV-associated neurocognitive disorders. Over the past two decades, a large number of papers have been published studying the molecular mechanisms of the involvement of α7 nAChRs in these two HIV-related processes as well as looking for ways to treat or mitigate the effects of infection, most of which have been considered in recent reviews (see, for example, [243]).

With the start of the COVID-19 pandemic, it was suggested that nAChRs might also be involved, although it was quickly shown that the main target for virus entry into the host cells is angiotensin-converting enzyme 2 (ACE2), which interacts with the receptor-binding domain (RBD) of the envelope Spike protein of the virus. The "cholinergic trace" arose after observing that smokers (that is, those who consume nicotine—an exogenous non-selective agonist of various nAChR subtypes) were infected less and more easily tolerated COVID-19 infection [244]. It was later shown that nicotine causes a decrease in cytokine levels, reducing cytokine storm, which is one of the main causes of mortality in COVID-19 infection [245,246], and also acting through α7 nAChR, can demonstrate other effects [247]. However, there is still no common view about the disease process in smoking and nonsmoking patients. Thus, some studies have shown the negative effect of smoking on the development and course of COVID-19 (see, for example, a recent review [248]). In particular, it was observed that smokers with COVID-19 had a higher risk of hospitalization and death [249]. One of the explanations is that nicotine enhances the expression of ACE2, facilitating the penetration and reproduction of the SARS-CoV-2 virus [250].

It has also been shown that α-bungarotoxin blocks the increase in the amount of ACE2 induced by the action of nicotine [251], mediated through α7 nAChR. Effects similar to those of nicotine were demonstrated by GTS-21, an agonist of α7 nAChR, which, unlike nicotine, is a more selective compound, does not have toxicity and does not cause addiction and, thus, can be considered a more-promising agent for the treatment of COVID-19 [252]. It is worth noting here that one of the first drugs used in the treatment of COVID-19 was chloroquine, which also interacts effectively with the α9α10 nAChR subtype [253]. Another area of research on the relationship of COVID-19 with the cholinergic system is the possibility

of direct interaction of nAChR with the S-protein of the SARS-CoV-2 virus. For the first time, such an assumption was made after the identification of a certain homology in some fragments of S-protein, with sequences of peptide antagonists of nAChRs, in particular, snake venom neurotoxins [254]. In one of the first such works, computer modeling of complexes of a number of nAChR subtypes and RBD was carried out, and the areas of their possible interaction were suggested. In particular, a high homology was revealed between the fragment (amino acid residues 375–390) of RBD of the SARS-CoV-2 virus and the snake neurotoxin NL1, which effectively interacts with nAChR [255]. In addition, according to the results of computer modeling, another region of RBD (381–386) was identified, which can form contact with the fragment (189–192) of the α9 subunit of the nAChR. Computer modeling also confirmed the high probability of the interaction of RBD with the α7 receptor subunit. Other "modelling" publications on the interaction of RBD with α7 nAChR [256] or various receptor subtypes have also appeared; for example [257], where possible, the recognition of the fragment (674–685) of the viral S-protein by three receptor subtypes-α4β2, α7 and α1β1$\gamma\delta$, was suggested. The experimental results concerning a possible interaction of the nAChR with the S protein fragments have appeared only very recently. In one publication [258], it was found that the immunization of mice with peptide 674–685 bound to hemocyanin resulted in decreased levels of α7 nAChRs and in higher levels of TNF-α and IL-1β. In more detail, the effects of the Y674-R685 peptide on α7 nAChRs were analyzed in [259]. Using whole-cell and single-channel recordings, its dual effects on this receptor were demonstrated: it activated the α7 nAChRs in the presence of positive allosteric modulators, thus indicating the attachment of this S-protein fragment in the vicinity of the agonist binding site but also induced a negative effect by a decrease in the duration of channel openings. However, in another recent publication [260], the authors tested the effects of the S1 domain and the entire S1–S2 ectodomain of S-protein but none of them were detected in terms of competition with radio-iodinated α-bungarotoxin for binding to the α7 nAChR heterologously expressed on the HEK-293 cells. The authors also did not see any effects of the S1 domain on the ion currents of this receptor. Apparently, further experiments are needed to elucidate a possible role, even a minor one, of nAChRs in COVID-19 disease.

8. Conclusions

The reviewed recent works in the field of nAChRs have shown increasing interest in the role that α7- and α9-containing receptors play in immune processes, chronic pain and viral infections. The presented materials demonstrated the occurrence and functional role of the mentioned nAChR subtypes in different immune cells. Nicotinic ligands, from small molecules to peptides such as α-conotoxins, as well as three-finger proteins, such as snake-venom α-neurotoxins and Ly6/uPAR proteins, are excellent tools in research on various nicotinic receptors, including α7- and α9-containing nAChRs, and are considered to be potential drug leads against diseases involving inflammation and pain.

In this review, we presented extensive information on the occurrence of α7- and α9-containing nAChRs in different kinds of immune cells, illustrated the participation of these receptor subtypes in various immune functions and also discussed versatile compounds affecting these receptors. Depending on the type of immune cells and the nAChR subtype they express, different biological effects can be observed. The role of nAChRs expressed by various types of immune cells described in this review may be useful for further drug development and therapeutic strategies for the treatment of immunopathological and inflammatory diseases. This information may also be useful in selecting appropriate tools for a more thorough study of immune system functions. To follow the second topic of this Special Issue, we provided a large amount of recent data on the involvement of these nAChRs in pain relief. In accordance with this, information was presented in sufficient detail on the selective compounds, such as three-finger proteins both from snake venoms and from the Ly6/uPAR family, as well as peptide neurotoxins from marine mollusks, which can open the way to new anesthetics. We hope that this information will be useful for researchers working in this and relevant directions.

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Abbreviations

ACE2	angiotensin-converting enzyme 2
ACh	acetylcholine
AChBP	acetylcholine-binding protein
APC	antigen-presenting cell
CAP	cholinergic anti-inflammatory pathway
CCI	chronic constriction injury
CCK	cholecystokinin
ChAT	choline acetyltransferase
CIA	collagen-induced arthritis
CIPN	chemotherapy-induced peripheral neuropathy
DC	dendritic cell
DRG	dorsal root ganglion
ECD	extracellular domain
GPI	glycosylphosphatidylinositol
HIV	human immunodeficiency virus
IFN	interferon
KO	knockout
MDM	human macrophage
MLA	methyllycaconitine
nAChR	nicotinic acetylcholine receptor
NK	natural killer
PHA	phytohemagglutinin
RVG	Rabies virus glycoprotein
RBD	receptor binding domain
SLURP-1	lymphocytic antigen-6/urokinase type plasminogen activator receptor-related peptide
Tregs	regulatory T-cells
wsLynx1	water-soluble Lynx1

References

1. Tsetlin, V.; Kuzmin, D.; Kasheverov, I. Assembly of nicotinic and other Cys-loop receptors. *J. Neurochem.* **2011**, *116*, 734–741. [CrossRef]
2. Dupont, Y.; Cohen, J.B.; Changeux, J.P. X-ray diffraction study of membrane fragments rich in acetylcholine receptor protein prepared from the electric organ of Torpedo marmorata. *FEBS Lett.* **1974**, *40*, 130–133. [CrossRef]
3. Unwin, N. The nicotinic acetylcholine receptor of the Torpedo electric ray. *J. Struct. Biol.* **1998**, *121*, 181–190. [CrossRef] [PubMed]
4. Brejc, K.; van Dijk, W.J.; Klaassen, R.V.; Schuurmans, M.; van Der Oost, J.; Smit, A.B.; Sixma, T.K. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* **2001**, *411*, 269–276. [CrossRef]
5. Unwin, N. Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J. Mol. Biol.* **2005**, *346*, 967–989. [CrossRef]
6. Rahman, M.M.; Teng, J.; Worrell, B.T.; Noviello, C.M.; Lee, M.; Karlin, A.; Stowell, M.H.B.; Hibbs, R.E. Structure of the Native Muscle-type Nicotinic Receptor and Inhibition by Snake Venom Toxins. *Neuron* **2020**, *106*, 952–962 e5. [CrossRef]
7. Nys, M.; Zarkadas, E.; Brams, M.; Mehregan, A.; Kambara, K.; Kool, J.; Casewell, N.R.; Bertrand, D.; Baenziger, J.E.; Nury, H.; et al. The molecular mechanism of snake short-chain α-neurotoxin binding to muscle-type nicotinic acetylcholine receptors. *Nat. Commun.* **2022**, *13*, 4543. [CrossRef]

8. Kabbani, N.; Nichols, R.A. Beyond the Channel: Metabotropic Signaling by Nicotinic Receptors. *Trends Pharmacol. Sci.* **2018**, *39*, 354–366. [CrossRef]

9. Bondarenko, V.; Wells, M.M.; Chen, Q.; Tillman, T.S.; Singewald, K.; Lawless, M.J.; Caporoso, J.; Brandon, N.; Coleman, J.A.; Saxena, S.; et al. Structures of highly flexible intracellular domain of human α7 nicotinic acetylcholine receptor. *Nat. Commun.* **2022**, *13*, 793. [CrossRef]

10. Utkin, Y.N.; Kukhtina, V.V.; Kryukova, E.V.; Chiodini, F.; Bertrand, D.; Methfessel, C.; Tsetlin, V.I. "Weak toxin" from Naja kaouthia is a nontoxic antagonist of α 7 and muscle-type nicotinic acetylcholine receptors. *J. Biol. Chem.* **2001**, *276*, 15810–15815. [CrossRef]

11. Shenkarev, Z.O.; Chesnokov, Y.M.; Zaigraev, M.M.; Chugunov, A.O.; Kulbatskii, D.S.; Kocharovskaya, M.V.; Paramonov, A.S.; Bychkov, M.L.; Shulepko, M.A.; Nolde, D.E.; et al. Membrane-mediated interaction of non-conventional snake three-finger toxins with nicotinic acetylcholine receptors. *Commun. Biol.* **2022**, *5*, 1344. [CrossRef]

12. Nirthanan, S.; Gopalakrishnakone, P.; Gwee, M.C.; Khoo, H.E.; Kini, R.M. Non-conventional toxins from Elapid venoms. *Toxicon Off. J. Int. Soc. Toxinol.* **2003**, *41*, 397–407. [CrossRef]

13. Miwa, J.M.; Ibanez-Tallon, I.; Crabtree, G.W.; Sanchez, R.; Sali, A.; Role, L.W.; Heintz, N. lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. *Neuron* **1999**, *23*, 105–114. [CrossRef]

14. Adermann, K.; Wattler, F.; Wattler, S.; Heine, G.; Meyer, M.; Forssmann, W.G.; Nehls, M. Structural and phylogenetic characterization of human SLURP-1, the first secreted mammalian member of the Ly-6/uPAR protein superfamily. *Protein Sci.* **1999**, *8*, 810–819. [CrossRef]

15. Chimienti, F.; Hogg, R.C.; Plantard, L.; Lehmann, C.; Brakch, N.; Fischer, J.; Huber, M.; Bertrand, D.; Hohl, D. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. *Hum. Mol. Genet.* **2003**, *12*, 3017–3024. [CrossRef]

16. Tsetlin, V.I. Three-finger snake neurotoxins and Ly6 proteins targeting nicotinic acetylcholine receptors: Pharmacological tools and endogenous modulators. *Trends Pharmacol. Sci.* **2015**, *36*, 109–123. [CrossRef]

17. Miwa, J.M.; Anderson, K.R.; Hoffman, K.M. Lynx Prototoxins: Roles of Endogenous Mammalian Neurotoxin-Like Proteins in Modulating Nicotinic Acetylcholine Receptor Function to Influence Complex Biological Processes. *Front. Pharmacol.* **2019**, *10*, 343. [CrossRef]

18. Tsetlin, V.I.; Kasheverov, I.E.; Utkin, Y.N. Three-finger proteins from snakes and humans acting on nicotinic receptors: Old and new. *J. Neurochem.* **2021**, *158*, 1223–1235. [CrossRef]

19. Lyukmanova, E.N.; Shenkarev, Z.O.; Shulepko, M.A.; Mineev, K.S.; D'Hoedt, D.; Kasheverov, I.E.; Filkin, S.Y.; Krivolapova, A.P.; Janickova, H.; Dolezal, V.; et al. NMR structure and action on nicotinic acetylcholine receptors of water-soluble domain of human LYNX1. *J. Biol. Chem.* **2011**, *286*, 10618–10627. [CrossRef]

20. Lyukmanova, E.N.; Shulepko, M.A.; Kudryavtsev, D.; Bychkov, M.L.; Kulbatskii, D.S.; Kasheverov, I.E.; Astapova, M.V.; Feofanov, A.V.; Thomsen, M.S.; Mikkelsen, J.D.; et al. Human Secreted Ly-6/uPAR Related Protein-1 (SLURP-1) Is a Selective Allosteric Antagonist of α7 Nicotinic Acetylcholine Receptor. *PLoS ONE* **2016**, *11*, e0149733. [CrossRef]

21. Durek, T.; Shelukhina, I.V.; Tae, H.S.; Thongyoo, P.; Spirova, E.N.; Kudryavtsev, D.S.; Kasheverov, I.E.; Faure, G.; Corringer, P.J.; Craik, D.J.; et al. Interaction of Synthetic Human SLURP-1 with the Nicotinic Acetylcholine Receptors. *Sci. Rep.* **2017**, *7*, 16606. [CrossRef]

22. Swamynathan, S.; Tiwari, A.; Loughner, C.L.; Gnalian, J.; Alexander, N.; Jhanji, V.; Swamynathan, S.K. The secreted Ly6/uPAR-related protein-1 suppresses neutrophil binding, chemotaxis, and transmigration through human umbilical vein endothelial cells. *Sci. Rep.* **2019**, *9*, 5898. [CrossRef]

23. Kawashima, K.; Fujii, T.; Moriwaki, Y.; Misawa, H.; Horiguchi, K. Non-neuronal cholinergic system in regulation of immune function with a focus on α7 nAChRs. *Int. Immunopharmacol.* **2015**, *29*, 127–134. [CrossRef]

24. Chernyavsky, A.I.; Galitovskiy, V.; Shchepotin, I.B.; Grando, S.A. Anti-inflammatory effects of the nicotinergic peptides SLURP-1 and SLURP-2 on human intestinal epithelial cells and immunocytes. *Biomed. Res. Int.* **2014**, *2014*, 609086. [CrossRef]

25. Campbell, G.; Swamynathan, S.; Tiwari, A.; Swamynathan, S.K. The secreted Ly-6/uPAR related protein-1 (SLURP-1) stabilizes epithelial cell junctions and suppresses TNF-α-induced cytokine production. *Biochem. Biophys. Res. Commun.* **2019**, *517*, 729–734. [CrossRef]

26. Ertle, C.M.; Rommel, F.R.; Tumala, S.; Moriwaki, Y.; Klein, J.; Kruse, J.; Gieler, U.; Peters, E.M.J. New Pathways for the Skin's Stress Response: The Cholinergic Neuropeptide SLURP-1 Can Activate Mast Cells and Alter Cytokine Production in Mice. *Front. Immunol.* **2021**, *12*, 631881. [CrossRef]

27. Paramonov, A.S.; Kocharovskaya, M.V.; Tsarev, A.V.; Kulbatskii, D.S.; Loktyushov, E.V.; Shulepko, M.A.; Kirpichnikov, M.P.; Lyukmanova, E.N.; Shenkarev, Z.O. Structural Diversity and Dynamics of Human Three-Finger Proteins Acting on Nicotinic Acetylcholine Receptors. *Int. J. Mol. Sci.* **2020**, *21*, 7280. [CrossRef]

28. Nissen, N.I.; Anderson, K.R.; Wang, H.; Lee, H.S.; Garrison, C.; Eichelberger, S.A.; Ackerman, K.; Im, W.; Miwa, J.M. Augmenting the antinociceptive effects of nicotinic acetylcholine receptor activity through lynx1 modulation. *PLoS ONE* **2018**, *13*, e0199643. [CrossRef]

29. Shenkarev, Z.O.; Shulepko, M.A.; Bychkov, M.L.; Kulbatskii, D.S.; Shlepova, O.V.; Vasilyeva, N.A.; Andreev-Andrievskiy, A.A.; Popova, A.S.; Lagereva, E.A.; Loktyushov, E.V.; et al. Water-soluble variant of human Lynx1 positively modulates synaptic plasticity and ameliorates cognitive impairment associated with α7-nAChR dysfunction. *J. Neurochem.* **2020**, *155*, 45–61. [CrossRef]

30. Kryukova, E.V.; Egorova, N.S.; Kudryavtsev, D.S.; Lebedev, D.S.; Spirova, E.N.; Zhmak, M.N.; Garifulina, A.I.; Kasheverov, I.E.; Utkin, Y.N.; Tsetlin, V.I. From Synthetic Fragments of Endogenous Three-Finger Proteins to Potential Drugs. *Front. Pharmacol.* **2019**, *10*, 748. [CrossRef]

31. Mineev, K.S.; Kryukova, E.V.; Kasheverov, I.E.; Egorova, N.S.; Zhmak, M.N.; Ivanov, I.A.; Senko, D.A.; Feofanov, A.V.; Ignatova, A.A.; Arseniev, A.S.; et al. Spatial Structure and Activity of Synthetic Fragments of Lynx1 and of Nicotinic Receptor Loop C Models. *Biomolecules* **2020**, *11*, 1. [CrossRef] [PubMed]

32. Throm, V.M.; Mannle, D.; Giese, T.; Bauer, A.S.; Gaida, M.M.; Kopitz, J.; Bruckner, T.; Plaschke, K.; Grekova, S.P.; Felix, K.; et al. Endogenous CHRNA7-ligand SLURP-1 as a potential tumor suppressor and anti-nicotinic factor in pancreatic cancer. *Oncotarget* **2018**, *9*, 11734–11751. [CrossRef] [PubMed]

33. Lyukmanova, E.N.; Bychkov, M.L.; Sharonov, G.V.; Efremenko, A.V.; Shulepko, M.A.; Kulbatskii, D.S.; Shenkarev, Z.O.; Feofanov, A.V.; Dolgikh, D.A.; Kirpichnikov, M.P. Human secreted proteins SLURP-1 and SLURP-2 control the growth of epithelial cancer cells via interactions with nicotinic acetylcholine receptors. *Br. J. Pharmacol.* **2018**, *175*, 1973–1986. [CrossRef]

34. Upadhyay, G. Emerging Role of Lymphocyte Antigen-6 Family of Genes in Cancer and Immune Cells. *Front. Immunol.* **2019**, *10*, 819. [CrossRef]

35. Shulepko, M.A.; Bychkov, M.L.; Shlepova, O.V.; Shenkarev, Z.O.; Kirpichnikov, M.P.; Lyukmanova, E.N. Human secreted protein SLURP-1 abolishes nicotine-induced proliferation, PTEN down-regulation and α7-nAChR expression up-regulation in lung cancer cells. *Int. Immunopharmacol.* **2020**, *82*, 106303. [CrossRef] [PubMed]

36. Lebbe, E.K.; Peigneur, S.; Wijesekara, I.; Tytgat, J. Conotoxins targeting nicotinic acetylcholine receptors: An overview. *Mar. Drugs* **2014**, *12*, 2970–3004. [CrossRef]

37. Abraham, N.; Lewis, R.J. Neuronal Nicotinic Acetylcholine Receptor Modulators from Cone Snails. *Mar. Drugs* **2018**, *16*, 208. [CrossRef]

38. Kasheverov, I.; Kudryavtsev, D.; Shelukhina, I.; Nikolaev, G.; Utkin, Y.; Tsetlin, V. Marine Origin Ligands of Nicotinic Receptors: Low Molecular Compounds, Peptides and Proteins for Fundamental Research and Practical Applications. *Biomolecules* **2022**, *12*, 189. [CrossRef]

39. Lin, B.; Xiang, S.; Li, M. Residues Responsible for the Selectivity of α-Conotoxins for Ac-AChBP or nAChRs. *Mar. Drugs* **2016**, *14*, 173. [CrossRef]

40. Ho, T.N.T.; Abraham, N.; Lewis, R.J. Structure-Function of Neuronal Nicotinic Acetylcholine Receptor Inhibitors Derived From Natural Toxins. *Front. Neurosci.* **2020**, *14*, 609005. [CrossRef]

41. Johnson, D.S.; Martinez, J.; Elgoyhen, A.B.; Heinemann, S.F.; McIntosh, J.M. α-Conotoxin ImI exhibits subtype-specific nicotinic acetylcholine receptor blockade: Preferential inhibition of homomeric α7 and α9 receptors. *Mol. Pharmacol.* **1995**, *48*, 194–199.

42. Broxton, N.M.; Down, J.G.; Gehrmann, J.; Alewood, P.F.; Satchell, D.G.; Livett, B.G. α-conotoxin ImI inhibits the α-bungarotoxin-resistant nicotinic response in bovine adrenal chromaffin cells. *J. Neurochem.* **1999**, *72*, 1656–1662. [CrossRef]

43. Ellison, M.; Gao, F.; Wang, H.L.; Sine, S.M.; McIntosh, J.M.; Olivera, B.M. α-conotoxins ImI and ImII target distinct regions of the human α7 nicotinic acetylcholine receptor and distinguish human nicotinic receptor subtypes. *Biochemistry* **2004**, *43*, 16019–16026. [CrossRef]

44. Ellison, M.; McIntosh, J.M.; Olivera, B.M. α-conotoxins ImI and ImII. Similar α7 nicotinic receptor antagonists act at different sites. *J. Biol. Chem.* **2003**, *278*, 757–764. [CrossRef] [PubMed]

45. Nicke, A.; Samochocki, M.; Loughnan, M.L.; Bansal, P.S.; Maelicke, A.; Lewis, R.J. α-conotoxins EpI and AuIB switch subtype selectivity and activity in native versus recombinant nicotinic acetylcholine receptors. *FEBS Lett.* **2003**, *554*, 219–223. [CrossRef] [PubMed]

46. Luo, S.; Nguyen, T.A.; Cartier, G.E.; Olivera, B.M.; Yoshikami, D.; McIntosh, J.M. Single-residue alteration in α-conotoxin PnIA switches its nAChR subtype selectivity. *Biochemistry* **1999**, *38*, 14542–14548. [CrossRef] [PubMed]

47. Loughnan, M.L.; Nicke, A.; Jones, A.; Adams, D.J.; Alewood, P.F.; Lewis, R.J. Chemical and functional identification and characterization of novel sulfated α-conotoxins from the cone snail Conus anemone. *J. Med. Chem.* **2004**, *47*, 1234–1241. [CrossRef] [PubMed]

48. Nicke, A.; Loughnan, M.L.; Millard, E.L.; Alewood, P.F.; Adams, D.J.; Daly, N.L.; Craik, D.J.; Lewis, R.J. Isolation, structure, and activity of GID, a novel α4/7-conotoxin with an extended N-terminal sequence. *J. Biol. Chem.* **2003**, *278*, 3137–3144. [CrossRef] [PubMed]

49. Talley, T.T.; Olivera, B.M.; Han, K.H.; Christensen, S.B.; Dowell, C.; Tsigelny, I.; Ho, K.Y.; Taylor, P.; McIntosh, J.M. α-conotoxin OmIA is a potent ligand for the acetylcholine-binding protein as well as α3β2 and α7 nicotinic acetylcholine receptors. *J. Biol. Chem.* **2006**, *281*, 24678–24686. [CrossRef]

50. Safavi-Hemami, H.; Siero, W.A.; Kuang, Z.; Williamson, N.A.; Karas, J.A.; Page, L.R.; MacMillan, D.; Callaghan, B.; Kompella, S.N.; Adams, D.J.; et al. Embryonic toxin expression in the cone snail Conus victoriae: Primed to kill or divergent function? *J. Biol. Chem.* **2011**, *286*, 22546–22557. [CrossRef]

51. Franco, A.; Kompella, S.N.; Akondi, K.B.; Melaun, C.; Daly, N.L.; Luetje, C.W.; Alewood, P.F.; Craik, D.J.; Adams, D.J.; Mari, F. RegIIA: An α4/7-conotoxin from the venom of Conus regius that potently blocks α3β4 nAChRs. *Biochem. Pharmacol.* **2012**, *83*, 419–426. [CrossRef] [PubMed]

52. Inserra, M.C.; Kompella, S.N.; Vetter, I.; Brust, A.; Daly, N.L.; Cuny, H.; Craik, D.J.; Alewood, P.F.; Adams, D.J.; Lewis, R.J. Isolation and characterization of α-conotoxin LsIA with potent activity at nicotinic acetylcholine receptors. *Biochem. Pharmacol.* **2013**, *86*, 791–799. [CrossRef] [PubMed]

53. Giribaldi, J.; Wilson, D.; Nicke, A.; El Hamdaoui, Y.; Laconde, G.; Faucherre, A.; Moha Ou Maati, H.; Daly, N.L.; Enjalbal, C.; Dutertre, S. Synthesis, Structure and Biological Activity of CIA and CIB, Two α-Conotoxins from the Predation-Evoked Venom of Conus catus. *Toxins* **2018**, *10*, 222. [CrossRef]

54. Tae, H.S.; Gao, B.; Jin, A.H.; Alewood, P.F.; Adams, D.J. Globular and ribbon isomers of Conus geographus α-conotoxins antagonize human nicotinic acetylcholine receptors. *Biochem. Pharmacol.* **2021**, *190*, 114638. [CrossRef] [PubMed]

55. Jin, A.H.; Vetter, I.; Dutertre, S.; Abraham, N.; Emidio, N.B.; Inserra, M.; Murali, S.S.; Christie, M.J.; Alewood, P.F.; Lewis, R.J. MrIC, a novel α-conotoxin agonist in the presence of PNU at endogenous α7 nicotinic acetylcholine receptors. *Biochemistry* **2014**, *53*, 1–3. [CrossRef]

56. Lebbe, E.K.; Peigneur, S.; Maiti, M.; Devi, P.; Ravichandran, S.; Lescrinier, E.; Ulens, C.; Waelkens, E.; D'Souza, L.; Herdewijn, P.; et al. Structure-function elucidation of a new α-conotoxin, Lo1a, from Conus longurionis. *J. Biol. Chem.* **2014**, *289*, 9573–9583. [CrossRef] [PubMed]

57. Nguyen, B.; Le Caer, J.P.; Araoz, R.; Thai, R.; Lamthanh, H.; Benoit, E.; Molgo, J. Isolation, purification and functional characterization of α-BnIA from Conus bandanus venom. *Toxicon* **2014**, *91*, 155–163. [CrossRef]

58. Lebbe, E.K.; Peigneur, S.; Maiti, M.; Mille, B.G.; Devi, P.; Ravichandran, S.; Lescrinier, E.; Waelkens, E.; D'Souza, L.; Herdewijn, P.; et al. Discovery of a new subclass of α-conotoxins in the venom of Conus australis. *Toxicon* **2014**, *91*, 145–154. [CrossRef]

59. Whiteaker, P.; Christensen, S.; Yoshikami, D.; Dowell, C.; Watkins, M.; Gulyas, J.; Rivier, J.; Olivera, B.M.; McIntosh, J.M. Discovery, synthesis, and structure activity of a highly selective α7 nicotinic acetylcholine receptor antagonist. *Biochemistry* **2007**, *46*, 6628–6638. [CrossRef]

60. Armishaw, C.J.; Singh, N.; Medina-Franco, J.L.; Clark, R.J.; Scott, K.C.; Houghten, R.A.; Jensen, A.A. A synthetic combinatorial strategy for developing α-conotoxin analogs as potent α7 nicotinic acetylcholine receptor antagonists. *J. Biol. Chem.* **2010**, *285*, 1809–1821. [CrossRef]

61. Kasheverov, I.E.; Chugunov, A.O.; Kudryavtsev, D.S.; Ivanov, I.A.; Zhmak, M.N.; Shelukhina, I.V.; Spirova, E.N.; Tabakmakher, V.M.; Zelepuga, E.A.; Efremov, R.G.; et al. High-Affinity α-Conotoxin PnIA Analogs Designed on the Basis of the Protein Surface Topography Method. *Sci. Rep.* **2016**, *6*, 36848. [CrossRef] [PubMed]

62. Kasheverov, I.E.; Zhmak, M.N.; Khruschov, A.Y.; Tsetlin, V.I. Design of new α-conotoxins: From computer modeling to synthesis of potent cholinergic compounds. *Mar. Drugs* **2011**, *9*, 1698–1714. [CrossRef] [PubMed]

63. Yu, J.; Zhu, X.; Zhang, L.; Kudryavtsev, D.; Kasheverov, I.; Lei, Y.; Zhangsun, D.; Tsetlin, V.; Luo, S. Species specificity of rat and human α7 nicotinic acetylcholine receptors towards different classes of peptide and protein antagonists. *Neuropharmacology* **2018**, *139*, 226–237. [CrossRef] [PubMed]

64. Wang, S.; Zhu, X.; Zhangsun, M.; Wu, Y.; Yu, J.; Harvey, P.J.; Kaas, Q.; Zhangsun, D.; Craik, D.J.; Luo, S. Engineered Conotoxin Differentially Blocks and Discriminates Rat and Human α7 Nicotinic Acetylcholine Receptors. *J. Med. Chem.* **2021**, *64*, 5620–5631. [CrossRef] [PubMed]

65. Innocent, N.; Livingstone, P.D.; Hone, A.; Kimura, A.; Young, T.; Whiteaker, P.; McIntosh, J.M.; Wonnacott, S. α-conotoxin Arenatus IB[V11L,V16D] [corrected] is a potent and selective antagonist at rat and human native α7 nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 529–537. [CrossRef]

66. Whiteaker, P.; Marks, M.J.; Christensen, S.; Dowell, C.; Collins, A.C.; McIntosh, J.M. Synthesis and characterization of 125I-α-conotoxin ArIB[V11L;V16A], a selective α7 nicotinic acetylcholine receptor antagonist. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 910–919. [CrossRef]

67. Hone, A.J.; Whiteaker, P.; Mohn, J.L.; Jacob, M.H.; McIntosh, J.M. Alexa Fluor 546-ArIB[V11L;V16A] is a potent ligand for selectively labeling α 7 nicotinic acetylcholine receptors. *J. Neurochem.* **2010**, *114*, 994–1006. [CrossRef]

68. Padilla, A.; Keating, P.; Hartmann, J.X.; Mari, F. Effects of α-conotoxin ImI on TNF-α, IL-8 and TGF-β expression by human macrophage-like cells derived from THP-1 pre-monocytic leukemic cells. *Sci. Rep.* **2017**, *7*, 12742. [CrossRef]

69. Grau, V.; Richter, K.; Hone, A.J.; McIntosh, J.M. Conopeptides [V11L;V16D]ArIB and RgIA4: Powerful Tools for the Identification of Novel Nicotinic Acetylcholine Receptors in Monocytes. *Front. Pharmacol.* **2018**, *9*, 1499. [CrossRef]

70. Tan, Y.; Chu, Z.; Shan, H.; Zhangsun, D.; Zhu, X.; Luo, S. Inflammation Regulation via an Agonist and Antagonists of α7 Nicotinic Acetylcholine Receptors in RAW264.7 Macrophages. *Mar. Drugs* **2022**, *20*, 200. [CrossRef]

71. Clark, R.J.; Fischer, H.; Nevin, S.T.; Adams, D.J.; Craik, D.J. The synthesis, structural characterization, and receptor specificity of the α-conotoxin Vc1.1. *J. Biol. Chem.* **2006**, *281*, 23254–23263. [CrossRef] [PubMed]

72. Vincler, M.; Wittenauer, S.; Parker, R.; Ellison, M.; Olivera, B.M.; McIntosh, J.M. Molecular mechanism for analgesia involving specific antagonism of α9α10 nicotinic acetylcholine receptors. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17880–17884. [CrossRef] [PubMed]

73. McIntosh, J.M.; Plazas, P.V.; Watkins, M.; Gomez-Casati, M.E.; Olivera, B.M.; Elgoyhen, A.B. A novel α-conotoxin, PeIA, cloned from Conus pergrandis, discriminates between rat α9α10 and α7 nicotinic cholinergic receptors. *J. Biol. Chem.* **2005**, *280*, 30107–30112. [CrossRef] [PubMed]

74. Ellison, M.; Haberlandt, C.; Gomez-Casati, M.E.; Watkins, M.; Elgoyhen, A.B.; McIntosh, J.M.; Olivera, B.M. α-RgIA: A novel conotoxin that specifically and potently blocks the α9α10 nAChR. *Biochemistry* **2006**, *45*, 1511–1517. [CrossRef] [PubMed]

75. Wang, S.; Zhao, C.; Liu, Z.; Wang, X.; Liu, N.; Du, W.; Dai, Q. Structural and Functional Characterization of a Novel α-Conotoxin Mr1.7 from Conus marmoreus Targeting Neuronal nAChR α3β2, α9α10 and α6/α3β2β3 Subtypes. *Mar. Drugs* **2015**, *13*, 3259–3275. [CrossRef] [PubMed]

76. Liang, J.; Tae, H.S.; Zhao, Z.; Li, X.; Zhang, J.; Chen, S.; Jiang, T.; Adams, D.J.; Yu, R. Mechanism of Action and Structure-Activity Relationship of α-Conotoxin Mr1.1 at the Human α9α10 Nicotinic Acetylcholine Receptor. *J. Med. Chem.* **2022**, *65*, 16204–16217. [CrossRef]

77. Zouridakis, M.; Papakyriakou, A.; Ivanov, I.A.; Kasheverov, I.E.; Tsetlin, V.; Tzartos, S.; Giastas, P. Crystal Structure of the Monomeric Extracellular Domain of α9 Nicotinic Receptor Subunit in Complex With α-Conotoxin RgIA: Molecular Dynamics Insights Into RgIA Binding to α9α10 Nicotinic Receptors. *Front. Pharmacol.* **2019**, *10*, 474. [CrossRef]

78. Satkunanathan, N.; Livett, B.; Gayler, K.; Sandall, D.; Down, J.; Khalil, Z. α-conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurones. *Brain Res.* **2005**, *1059*, 149–158. [CrossRef]

79. Nevin, S.T.; Clark, R.J.; Klimis, H.; Christie, M.J.; Craik, D.J.; Adams, D.J. Are α9α10 nicotinic acetylcholine receptors a pain target for α-conotoxins? *Mol. Pharmacol.* **2007**, *72*, 1406–1410. [CrossRef]

80. Mohammadi, S.A.; Christie, M.J. Conotoxin Interactions with α9α10-nAChRs: Is the α9α10-Nicotinic Acetylcholine Receptor an Important Therapeutic Target for Pain Management? *Toxins* **2015**, *7*, 3916–3932. [CrossRef] [PubMed]

81. Carstens, B.B.; Berecki, G.; Daniel, J.T.; Lee, H.S.; Jackson, K.A.; Tae, H.S.; Sadeghi, M.; Castro, J.; O'Donnell, T.; Deiteren, A.; et al. Structure-Activity Studies of Cysteine-Rich α-Conotoxins that Inhibit High-Voltage-Activated Calcium Channels via GABA(B) Receptor Activation Reveal a Minimal Functional Motif. *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 4692–4696. [CrossRef] [PubMed]

82. Azam, L.; McIntosh, J.M. Molecular basis for the differential sensitivity of rat and human α9α10 nAChRs to α-conotoxin RgIA. *J. Neurochem.* **2012**, *122*, 1137–1144. [CrossRef] [PubMed]

83. Yu, R.; Kompella, S.N.; Adams, D.J.; Craik, D.J.; Kaas, Q. Determination of the α-conotoxin Vc1.1 binding site on the α9α10 nicotinic acetylcholine receptor. *J. Med. Chem.* **2013**, *56*, 3557–3567. [CrossRef] [PubMed]

84. Chu, X.; Tae, H.S.; Xu, Q.; Jiang, T.; Adams, D.J.; Yu, R. α-Conotoxin Vc1.1 Structure-Activity Relationship at the Human α9α10 Nicotinic Acetylcholine Receptor Investigated by Minimal Side Chain Replacement. *ACS Chem. Neurosci.* **2019**, *10*, 4328–4336. [CrossRef]

85. Romero, H.K.; Christensen, S.B.; Di Cesare Mannelli, L.; Gajewiak, J.; Ramachandra, R.; Elmslie, K.S.; Vetter, D.E.; Ghelardini, C.; Iadonato, S.P.; Mercado, J.L.; et al. Inhibition of α9α10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E1825–E1832. [CrossRef]

86. Christensen, S.B.; Hone, A.J.; Roux, I.; Kniazeff, J.; Pin, J.P.; Upert, G.; Servent, D.; Glowatzki, E.; McIntosh, J.M. RgIA4 Potently Blocks Mouse α9α10 nAChRs and Provides Long Lasting Protection against Oxaliplatin-Induced Cold Allodynia. *Front. Cell. Neurosci.* **2017**, *11*, 219. [CrossRef]

87. Gajewiak, J.; Christensen, S.B.; Dowell, C.; Hararah, F.; Fisher, F.; Huynh, P.N.; Olivera, B.M.; McIntosh, J.M. Selective Penicillamine Substitution Enables Development of a Potent Analgesic Peptide that Acts through a Non-Opioid-Based Mechanism. *J. Med. Chem.* **2021**, *64*, 9271–9278. [CrossRef]

88. Huynh, P.N.; Giuvelis, D.; Christensen, S.; Tucker, K.L.; McIntosh, J.M. RgIA4 Accelerates Recovery from Paclitaxel-Induced Neuropathic Pain in Rats. *Mar. Drugs* **2019**, *18*, 12. [CrossRef]

89. Luo, S.; Zhangsun, D.; Harvey, P.J.; Kaas, Q.; Wu, Y.; Zhu, X.; Hu, Y.; Li, X.; Tsetlin, V.I.; Christensen, S.; et al. Cloning, synthesis, and characterization of αO-conotoxin GeXIVA, a potent α9α10 nicotinic acetylcholine receptor antagonist. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E4026–E4035. [CrossRef]

90. Jiang, S.; Tae, H.S.; Xu, S.; Shao, X.; Adams, D.J.; Wang, C. Identification of a Novel O-Conotoxin Reveals an Unusual and Potent Inhibitor of the Human α9α10 Nicotinic Acetylcholine Receptor. *Mar. Drugs* **2017**, *15*, 170. [CrossRef]

91. Christensen, S.B.; Bandyopadhyay, P.K.; Olivera, B.M.; McIntosh, J.M. αS-conotoxin GVIIIB potently and selectively blocks α9α10 nicotinic acetylcholine receptors. *Biochem. Pharmacol.* **2015**, *96*, 349–356. [CrossRef] [PubMed]

92. Lu, J.; Zhang, K.; Wang, S.; Sun, T.; Yu, S.; Dai, Q.; Liu, Z. Cloning, expression and functional characterization of a D-superfamily conotoxin Lt28.1 with previously undescribed cysteine pattern. *Peptides* **2017**, *94*, 64–70. [CrossRef] [PubMed]

93. Wang, H.; Li, X.; Zhangsun, D.; Yu, G.; Su, R.; Luo, S. The α9α10 Nicotinic Acetylcholine Receptor Antagonist αO-Conotoxin GeXIVA[1,2] Alleviates and Reverses Chemotherapy-Induced Neuropathic Pain. *Mar. Drugs* **2019**, *17*, 265. [CrossRef] [PubMed]

94. Li, X.; Hu, Y.; Wu, Y.; Huang, Y.; Yu, S.; Ding, Q.; Zhangsun, D.; Luo, S. Anti-hypersensitive effect of intramuscular administration of αO-conotoxin GeXIVA[1,2] and GeXIVA[1,4] in rats of neuropathic pain. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2016**, *66*, 112–119. [CrossRef]

95. Lebedev, D.S.; Kryukova, E.V.; Ivanov, I.A.; Egorova, N.S.; Timofeev, N.D.; Spirova, E.N.; Tufanova, E.Y.; Siniavin, A.E.; Kudryavtsev, D.S.; Kasheverov, I.E.; et al. Oligoarginine Peptides, a New Family of Nicotinic Acetylcholine Receptor Inhibitors. *Mol. Pharmacol.* **2019**, *96*, 664–673. [CrossRef]

96. Zhang, B.; Ren, M.; Yang, F.; Li, R.; Yu, L.; Luo, A.; Zhangsun, D.; Luo, S.; Dong, S. Oligo-basic amino acids, potential nicotinic acetylcholine receptor inhibitors. *Biomed. Pharmacother.* **2022**, *152*, 113215. [CrossRef]

97. MacDougall, G.; Anderton, R.S.; Edwards, A.B.; Knuckey, N.W.; Meloni, B.P. The Neuroprotective Peptide Poly-Arginine-12 (R12) Reduces Cell Surface Levels of NMDA NR2B Receptor Subunit in Cortical Neurons; Investigation into the Involvement of Endocytic Mechanisms. *J. Mol. Neurosci.* **2017**, *61*, 235–246. [CrossRef]

98. Kelly, M.J.; Breathnach, C.; Tracey, K.J.; Donnelly, S.C. Manipulation of the inflammatory reflex as a therapeutic strategy. *Cell Rep. Med.* **2022**, *3*, 100696. [CrossRef]

99. Borovikova, L.V.; Ivanova, S.; Zhang, M.; Yang, H.; Botchkina, G.I.; Watkins, L.R.; Wang, H.; Abumrad, N.; Eaton, J.W.; Tracey, K.J. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* **2000**, *405*, 458–462. [CrossRef]

100. Tracey, K.J. The inflammatory reflex. *Nature* **2002**, *420*, 853–859. [CrossRef]

101. Sansonetti, P.J. The innate signaling of dangers and the dangers of innate signaling. *Nat. Immunol.* **2006**, *7*, 1237–1242. [CrossRef] [PubMed]

102. Luyer, M.D.; Greve, J.W.; Hadfoune, M.; Jacobs, J.A.; Dejong, C.H.; Buurman, W.A. Nutritional stimulation of cholecystokinin receptors inhibits inflammation via the vagus nerve. *J. Exp. Med.* **2005**, *202*, 1023–1029. [CrossRef] [PubMed]

103. de Jonge, W.J.; van der Zanden, E.P.; The, F.O.; Bijlsma, M.F.; van Westerloo, D.J.; Bennink, R.J.; Berthoud, H.R.; Uematsu, S.; Akira, S.; van den Wijngaard, R.M.; et al. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat. Immunol.* **2005**, *6*, 844–851. [CrossRef]

104. Parameswaran, N.; Patial, S. Tumor necrosis factor-α signaling in macrophages. *Crit. Rev. Eukaryot. Gene Expr.* **2010**, *20*, 87–103. [CrossRef] [PubMed]

105. Wang, H.; Yu, M.; Ochani, M.; Amella, C.A.; Tanovic, M.; Susarla, S.; Li, J.H.; Wang, H.; Yang, H.; Ulloa, L.; et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature* **2003**, *421*, 384–388. [CrossRef] [PubMed]

106. Rosas-Ballina, M.; Ochani, M.; Parrish, W.R.; Ochani, K.; Harris, Y.T.; Huston, J.M.; Chavan, S.; Tracey, K.J. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11008–11013. [CrossRef]

107. Vida, G.; Pena, G.; Deitch, E.A.; Ulloa, L. α7-cholinergic receptor mediates vagal induction of splenic norepinephrine. *J. Immunol.* **2011**, *186*, 4340–4346. [CrossRef]

108. Huston, J.M.; Ochani, M.; Rosas-Ballina, M.; Liao, H.; Ochani, K.; Pavlov, V.A.; Gallowitsch-Puerta, M.; Ashok, M.; Czura, C.J.; Foxwell, B.; et al. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J. Exp. Med.* **2006**, *203*, 1623–1628. [CrossRef]

109. Vida, G.; Pena, G.; Kanashiro, A.; Thompson-Bonilla Mdel, R.; Palange, D.; Deitch, E.A.; Ulloa, L. β2-Adrenoreceptors of regulatory lymphocytes are essential for vagal neuromodulation of the innate immune system. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2011**, *25*, 4476–4485. [CrossRef]

110. Rosas-Ballina, M.; Olofsson, P.S.; Ochani, M.; Valdes-Ferrer, S.I.; Levine, Y.A.; Reardon, C.; Tusche, M.W.; Pavlov, V.A.; Andersson, U.; Chavan, S.; et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* **2011**, *334*, 98–101. [CrossRef]

111. Vieira-Alves, I.; Coimbra-Campos, L.M.C.; Sancho, M.; da Silva, R.F.; Cortes, S.F.; Lemos, V.S. Role of the α7 Nicotinic Acetylcholine Receptor in the Pathophysiology of Atherosclerosis. *Front. Physiol.* **2020**, *11*, 621769. [CrossRef] [PubMed]

112. Zoheir, N.; Lappin, D.F.; Nile, C.J. Acetylcholine and the α 7 nicotinic receptor: A potential therapeutic target for the treatment of periodontal disease? *Inflamm. Res. Off. J. Eur. Histamine Res. Soc.* **2012**, *61*, 915–926. [CrossRef] [PubMed]

113. Fujii, T.; Mashimo, M.; Moriwaki, Y.; Misawa, H.; Ono, S.; Horiguchi, K.; Kawashima, K. Physiological functions of the cholinergic system in immune cells. *J. Pharmacol. Sci.* **2017**, *134*, 1–21. [CrossRef] [PubMed]

114. Fujii, T.; Tsuchiya, T.; Yamada, S.; Fujimoto, K.; Suzuki, T.; Kasahara, T.; Kawashima, K. Localization and synthesis of acetylcholine in human leukemic T cell lines. *J. Neurosci. Res.* **1996**, *44*, 66–72. [CrossRef]

115. Fujii, T.; Yamada, S.; Watanabe, Y.; Misawa, H.; Tajima, S.; Fujimoto, K.; Kasahara, T.; Kawashima, K. Induction of choline acetyltransferase mRNA in human mononuclear leukocytes stimulated by phytohemagglutinin, a T-cell activator. *J. Neuroimmunol.* **1998**, *82*, 101–107. [CrossRef]

116. Peng, H.; Ferris, R.L.; Matthews, T.; Hiel, H.; Lopez-Albaitero, A.; Lustig, L.R. Characterization of the human nicotinic acetylcholine receptor subunit α (α) 9 (CHRNA9) and α (α) 10 (CHRNA10) in lymphocytes. *Life Sci.* **2004**, *76*, 263–280. [CrossRef]

117. Fujii, T.; Mashimo, M.; Moriwaki, Y.; Misawa, H.; Ono, S.; Horiguchi, K.; Kawashima, K. Expression and Function of the Cholinergic System in Immune Cells. *Front. Immunol.* **2017**, *8*, 1085. [CrossRef]

118. Willemze, R.A.; Brinkman, D.J.; Welting, O.; van Hamersveld, P.H.P.; Verseijden, C.; Luyer, M.D.; Wildenberg, M.E.; Seppen, J.; de Jonge, W.J. Acetylcholine-producing T cells augment innate immune-driven colitis but are redundant in T cell-driven colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2019**, *317*, G557–G568. [CrossRef]

119. Fujii, Y.X.; Fujigaya, H.; Moriwaki, Y.; Misawa, H.; Kasahara, T.; Grando, S.A.; Kawashima, K. Enhanced serum antigen-specific IgG1 and proinflammatory cytokine production in nicotinic acetylcholine receptor α7 subunit gene knockout mice. *J. Neuroimmunol.* **2007**, *189*, 69–74. [CrossRef]

120. Oloris, S.C.; Frazer-Abel, A.A.; Jubala, C.M.; Fosmire, S.P.; Helm, K.M.; Robinson, S.R.; Korpela, D.M.; Duckett, M.M.; Baksh, S.; Modiano, J.F. Nicotine-mediated signals modulate cell death and survival of T lymphocytes. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 299–309. [CrossRef]

121. Nouri-Shirazi, M.; Guinet, E. Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. *Immunology* **2003**, *109*, 365–373. [CrossRef] [PubMed]

122. Mashimo, M.; Komori, M.; Matsui, Y.Y.; Murase, M.X.; Fujii, T.; Takeshima, S.; Okuyama, H.; Ono, S.; Moriwaki, Y.; Misawa, H.; et al. Distinct Roles of α7 nAChRs in Antigen-Presenting Cells and CD4(+) T Cells in the Regulation of T Cell Differentiation. *Front. Immunol.* **2019**, *10*, 1102. [CrossRef]

123. Nizri, E.; Irony-Tur-Sinai, M.; Lory, O.; Orr-Utrreger, A.; Lavi, E.; Brenner, T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. *J. Immunol.* **2009**, *183*, 6681–6688. [CrossRef]

124. Wang, D.W.; Zhou, R.B.; Yao, Y.M.; Zhu, X.M.; Yin, Y.M.; Zhao, G.J.; Dong, N.; Sheng, Z.Y. Stimulation of α7 nicotinic acetylcholine receptor by nicotine increases suppressive capacity of naturally occurring $CD_4^+CD_{25}^+$ regulatory T cells in mice in vitro. *J. Pharm. Exp. Ther.* **2010**, *335*, 553–561. [CrossRef]

125. Nakata, Y.; Miura, K.; Yamasaki, N.; Ogata, S.; Miura, S.; Hosomi, N.; Kaminuma, O. Expression and Function of Nicotinic Acetylcholine Receptors in Induced Regulatory T Cells. *Int. J. Mol. Sci.* **2022**, *23*, 1779. [CrossRef]

126. Zhao, X.; Wilson, K.; Uteshev, V.; He, J.J. Activation of α7 nicotinic acetylcholine receptor ameliorates HIV-associated neurology and neuropathology. *Brain* **2021**, *144*, 3355–3370. [CrossRef] [PubMed]

127. Reardon, C.; Duncan, G.S.; Brustle, A.; Brenner, D.; Tusche, M.W.; Olofsson, P.S.; Rosas-Ballina, M.; Tracey, K.J.; Mak, T.W. Lymphocyte-derived ACh regulates local innate but not adaptive immunity. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 1410–1415. [CrossRef] [PubMed]

128. Skok, M.V.; Grailhe, R.; Agenes, F.; Changeux, J.P. The role of nicotinic receptors in B-lymphocyte development and activation. *Life Sci.* **2007**, *80*, 2334–2336. [CrossRef]

129. Skok, M.; Grailhe, R.; Agenes, F.; Changeux, J.P. The role of nicotinic acetylcholine receptors in lymphocyte development. *J. Neuroimmunol.* **2006**, *171*, 86–98. [CrossRef]

130. Skok, M.; Grailhe, R.; Changeux, J.P. Nicotinic receptors regulate B lymphocyte activation and immune response. *Eur. J. Pharm.* **2005**, *517*, 246–251. [CrossRef]

131. Koval, L.; Kalashnyk, O.; Lykhmus, O.; Skok, M. α7 nicotinic acetylcholine receptors are involved in suppression of the antibody immune response. *J. Neuroimmunol.* **2018**, *318*, 8–14. [CrossRef] [PubMed]

132. Ness, S.; Lin, S.; Gordon, J.R. Regulatory Dendritic Cells, T Cell Tolerance, and Dendritic Cell Therapy for Immunologic Disease. *Front. Immunol.* **2021**, *12*, 633436. [CrossRef] [PubMed]

133. Hogg, N. Nicotine has suppressive effects on dendritic cell function. *Immunology* **2003**, *109*, 329–330. [CrossRef]

134. Fujii, T.; Horiguchi, K.; Sunaga, H.; Moriwaki, Y.; Misawa, H.; Kasahara, T.; Tsuji, S.; Kawashima, K. SLURP-1, an endogenous α7 nicotinic acetylcholine receptor allosteric ligand, is expressed in CD205(+) dendritic cells in human tonsils and potentiates lymphocytic cholinergic activity. *J. Neuroimmunol.* **2014**, *267*, 43–49. [CrossRef] [PubMed]

135. Liu, D.; Li, T.; Luo, H.; Zuo, X.; Liu, S.; Wu, S. The effect of the cholinergic anti-inflammatory pathway on collagen-induced arthritis involves the modulation of dendritic cell differentiation. *Arthritis Res. Ther.* **2018**, *20*, 263. [CrossRef]

136. Mashimo, M.; Takeshima, S.; Okuyama, H.; Matsurida, A.; Murase, M.; Ono, S.; Kawashima, K.; Fujii, T. α7 nAChRs expressed on antigen presenting cells are insensitive to the conventional antagonists α-bungarotoxin and methyllycaconitine. *Int. Immunopharmacol.* **2020**, *81*, 106276. [CrossRef]

137. Gori, S.; Vermeulen, M.; Remes-Lenicov, F.; Jancic, C.; Scordo, W.; Ceballos, A.; Towstyka, N.; Bestach, Y.; Belli, C.; Sabbione, F.; et al. Acetylcholine polarizes dendritic cells toward a Th2-promoting profile. *Allergy* **2017**, *72*, 221–231. [CrossRef]

138. Nouri-Shirazi, M.; Kahlden, C.; Nishino, P.; Guinet, E. Nicotine exposure alters the mRNA expression of Notch ligands in dendritic cells and their response to Th1-/Th2-promoting stimuli. *Scand. J. Immunol.* **2015**, *81*, 110–120. [CrossRef]

139. Li, R.; Hu, X.; Chen, H.; Zhao, Y.; Gao, X.; Yuan, Y.; Guo, H.; Huang, H.; Zou, X.; Qi, H.; et al. Role of Cholinergic Anti-Inflammatory Pathway in Protecting Sepsis-Induced Acute Lung Injury through Regulation of the Conventional Dendritic Cells. *Mediat. Inflamm.* **2022**, *2022*, 1474891. [CrossRef]

140. Chernyavsky, A.I.; Arredondo, J.; Skok, M.; Grando, S.A. Auto/paracrine control of inflammatory cytokines by acetylcholine in macrophage-like U937 cells through nicotinic receptors. *Int. Immunopharmacol.* **2010**, *10*, 308–315. [CrossRef]

141. van der Zanden, E.P.; Hilbers, F.W.; Verseijden, C.; van den Wijngaard, R.M.; Skynner, M.; Lee, K.; Ulloa, L.; Boeckxstaens, G.E.; de Jonge, W.J. Nicotinic acetylcholine receptor expression and susceptibility to cholinergic immunomodulation in human monocytes of smoking individuals. *Neuroimmunomodulation* **2012**, *19*, 255–265. [CrossRef] [PubMed]

142. Takahashi, H.K.; Iwagaki, H.; Hamano, R.; Yoshino, T.; Tanaka, N.; Nishibori, M. Effect of nicotine on IL-18-initiated immune response in human monocytes. *J. Leukoc. Biol.* **2006**, *80*, 1388–1394. [CrossRef]

143. Takahashi, H.K.; Iwagaki, H.; Hamano, R.; Yoshino, T.; Tanaka, N.; Nishibori, M. α7 Nicotinic acetylcholine receptor stimulation inhibits lipopolysaccharide-induced interleukin-18 and -12 production in monocytes. *J. Pharmacol. Sci.* **2006**, *102*, 143–146. [CrossRef] [PubMed]

144. Yang, X.; Zhao, C.; Chen, X.; Jiang, L.; Su, X. Monocytes primed with GTS-21/α7 nAChR (nicotinic acetylcholine receptor) agonist develop anti-inflammatory memory. *QJM Mon. J. Assoc. Physicians* **2017**, *110*, 437–445. [CrossRef] [PubMed]

145. St-Pierre, S.; Jiang, W.; Roy, P.; Champigny, C.; LeBlanc, E.; Morley, B.J.; Hao, J.; Simard, A.R. Nicotinic Acetylcholine Receptors Modulate Bone Marrow-Derived Pro-Inflammatory Monocyte Production and Survival. *PLoS ONE* **2016**, *11*, e0150230. [CrossRef]

146. Jiang, W.; St-Pierre, S.; Roy, P.; Morley, B.J.; Hao, J.; Simard, A.R. Infiltration of CCR2+Ly6Chigh Proinflammatory Monocytes and Neutrophils into the Central Nervous System Is Modulated by Nicotinic Acetylcholine Receptors in a Model of Multiple Sclerosis. *J. Immunol.* **2016**, *196*, 2095–2108. [CrossRef]

147. Hu, J.N.; Liu, Y.; Liu, S.C.; Zhang, T.; Chen, G.B.; Zhao, J.; Ma, T. The α7 Nicotinic Acetylcholine Receptor Agonist GTS-21 Improves Bacterial Clearance via Regulation of Monocyte Recruitment and Activity in Polymicrobial Septic Peritonitis. *Front. Immunol.* **2022**, *13*, 839290. [CrossRef]

148. Richter, K.; Mathes, V.; Fronius, M.; Althaus, M.; Hecker, A.; Krasteva-Christ, G.; Padberg, W.; Hone, A.J.; McIntosh, J.M.; Zakrzewicz, A.; et al. Phosphocholine—An agonist of metabotropic but not of ionotropic functions of α9-containing nicotinic acetylcholine receptors. *Sci. Rep.* **2016**, *6*, 28660. [CrossRef]

149. Zakrzewicz, A.; Richter, K.; Agne, A.; Wilker, S.; Siebers, K.; Fink, B.; Krasteva-Christ, G.; Althaus, M.; Padberg, W.; Hone, A.J.; et al. Canonical and Novel Non-Canonical Cholinergic Agonists Inhibit ATP-Induced Release of Monocytic Interleukin-1β via Different Combinations of Nicotinic Acetylcholine Receptor Subunits α7, α9 and α10. *Front. Cell. Neurosci.* **2017**, *11*, 189. [CrossRef]

150. Backhaus, S.; Zakrzewicz, A.; Richter, K.; Damm, J.; Wilker, S.; Fuchs-Moll, G.; Kullmar, M.; Hecker, A.; Manzini, I.; Ruppert, C.; et al. Surfactant inhibits ATP-induced release of interleukin-1β via nicotinic acetylcholine receptors. *J. Lipid Res.* **2017**, *58*, 1055–1066. [CrossRef]

151. Hecker, A.; Kullmar, M.; Wilker, S.; Richter, K.; Zakrzewicz, A.; Atanasova, S.; Mathes, V.; Timm, T.; Lerner, S.; Klein, J.; et al. Phosphocholine-Modified Macromolecules and Canonical Nicotinic Agonists Inhibit ATP-Induced IL-1β Release. *J. Immunol.* **2015**, *195*, 2325–2334. [CrossRef] [PubMed]

152. Richter, K.; Sagawe, S.; Hecker, A.; Kullmar, M.; Askevold, I.; Damm, J.; Heldmann, S.; Pohlmann, 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. [CrossRef] [PubMed]

153. Richter, K.; Koch, C.; Perniss, A.; Wolf, P.M.; Schweda, E.K.H.; Wichmann, S.; Wilker, S.; Magel, I.; Sander, M.; McIntosh, J.M.; et al. Phosphocholine-Modified Lipooligosaccharides of Haemophilus influenzae Inhibit ATP-Induced IL-1β Release by Pulmonary Epithelial Cells. *Molecules* **2018**, *23*, 1979. [CrossRef] [PubMed]

154. Mikulski, Z.; Hartmann, P.; Jositsch, G.; Zaslona, Z.; Lips, K.S.; Pfeil, U.; Kurzen, H.; Lohmeyer, J.; Clauss, W.G.; Grau, V.; et al. Nicotinic receptors on rat alveolar macrophages dampen ATP-induced increase in cytosolic calcium concentration. *Respir. Res.* **2010**, *11*, 133. [CrossRef]

155. Tarnawski, L.; Reardon, C.; Caravaca, A.S.; Rosas-Ballina, M.; Tusche, M.W.; Drake, A.R.; Hudson, L.K.; Hanes, W.M.; Li, J.H.; Parrish, W.R.; et al. Adenylyl Cyclase 6 Mediates Inhibition of TNF in the Inflammatory Reflex. *Front. Immunol.* **2018**, *9*, 2648. [CrossRef]

156. Siniavin, A.E.; Streltsova, M.A.; Kudryavtsev, D.S.; Shelukhina, I.V.; Utkin, Y.N.; Tsetlin, V.I. Activation of α7 Nicotinic Acetyl-choline Receptor Upregulates HLA-DR and Macrophage Receptors: Potential Role in Adaptive Immunity and in Preventing Immunosuppression. *Biomolecules* **2020**, *10*, 507. [CrossRef]

157. Yang, Y.H.; Li, D.L.; Bi, X.Y.; Sun, L.; Yu, X.J.; Fang, H.L.; Miao, Y.; Zhao, M.; He, X.; Liu, J.J.; et al. Acetylcholine Inhibits LPS-Induced MMP-9 Production and Cell Migration via the α7 nAChR-JAK2/STAT3 Pathway in RAW264.7 Cells. *Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* **2015**, *36*, 2025–2038. [CrossRef]

158. Su, Y.; Zhang, W.; Zhang, R.; Yuan, Q.; Wu, R.; Liu, X.; Wuri, J.; Li, R.; Yan, T. Activation of Cholinergic Anti-Inflammatory Pathway Ameliorates Cerebral and Cardiac Dysfunction After Intracerebral Hemorrhage Through Autophagy. *Front. Immunol.* **2022**, *13*, 870174. [CrossRef]

159. Su, X.; Lee, J.W.; Matthay, Z.A.; Mednick, G.; Uchida, T.; Fang, X.; Gupta, N.; Matthay, M.A. Activation of the α7 nAChR reduces acid-induced acute lung injury in mice and rats. *Am. J. Respir. Cell Mol. Biol.* **2007**, *37*, 186–192. [CrossRef]

160. Giebelen, I.A.; van Westerloo, D.J.; LaRosa, G.J.; de Vos, A.F.; van der Poll, T. Local stimulation of α7 cholinergic receptors inhibits LPS-induced TNF-α release in the mouse lung. *Shock* **2007**, *28*, 700–703. [CrossRef]

161. Delgado-Velez, M.; Baez-Pagan, C.A.; Gerena, Y.; Quesada, O.; Santiago-Perez, L.I.; Capo-Velez, C.M.; Wojna, V.; Melendez, L.; Leon-Rivera, R.; Silva, W.; et al. The α7-nicotinic receptor is upregulated in immune cells from HIV-seropositive women: Consequences to the cholinergic anti-inflammatory response. *Clin. Transl. Immunol.* **2015**, *4*, e53. [CrossRef]

162. Rios, S.C.; Colon Saez, J.O.; Quesada, O.; Figueroa, K.Q.; Lasalde Dominicci, J.A. Disruption of the cholinergic anti-inflammatory response by R5-tropic HIV-1 protein gp120(JRFL). *J. Biol. Chem.* **2021**, *296*, 100618. [CrossRef] [PubMed]

163. Mishra, N.C.; Rir-sima-ah, J.; Boyd, R.T.; Singh, S.P.; Gundavarapu, S.; Langley, R.J.; Razani-Boroujerdi, S.; Sopori, M.L. Nicotine inhibits Fc epsilon RI-induced cysteinyl leukotrienes and cytokine production without affecting mast cell degranulation through α 7/α 9/α 10-nicotinic receptors. *J. Immunol.* **2010**, *185*, 588–596. [CrossRef]

164. Fantozzi, R.; Masini, E.; Blandina, P.; Mannaioni, P.F.; Bani-Sacchi, T. Release of histamine from rat mast cells by acetylcholine. *Nature* **1978**, *273*, 473–474. [CrossRef] [PubMed]

165. Sudheer, P.S.; Hall, J.E.; Donev, R.; Read, G.; Rowbottom, A.; Williams, P.E. Nicotinic acetylcholine receptors on basophils and mast cells. *Anaesthesia* **2006**, *61*, 1170–1174. [CrossRef] [PubMed]

166. Radosa, J.; Dyck, W.; Goerdt, S.; Kurzen, H. The cholinergic system in guttate psoriasis with special reference to mast cells. *Exp. Dermatol.* **2011**, *20*, 677–679. [CrossRef]

167. Kageyama-Yahara, N.; Suehiro, Y.; Yamamoto, T.; Kadowaki, M. IgE-induced degranulation of mucosal mast cells is negatively regulated via nicotinic acetylcholine receptors. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 321–325. [CrossRef]

168. Safronova, V.G.; Vulfius, C.A.; Shelukhina, I.V.; Mal'tseva, V.N.; Berezhnov, A.V.; Fedotova, E.I.; Miftahova, R.G.; Kryukova, E.V.; Grinevich, A.A.; Tsetlin, V.I. Nicotinic receptor involvement in regulation of functions of mouse neutrophils from inflammatory site. *Immunobiology* **2016**, *221*, 761–772. [CrossRef]

169. Safronova, V.G.; Vulfius, C.A.; Astashev, M.E.; Tikhonova, I.V.; Serov, D.A.; Jirova, E.A.; Pershina, E.V.; Senko, D.A.; Zhmak, M.N.; Kasheverov, I.E.; et al. α9α10 nicotinic acetylcholine receptors regulate murine bone marrow granulocyte functions. *Immunobiology* **2021**, *226*, 152047. [CrossRef]

170. Hosseinzadeh, A.; Thompson, P.R.; Segal, B.H.; Urban, C.F. Nicotine induces neutrophil extracellular traps. *J. Leukoc. Biol.* **2016**, *100*, 1105–1112. [CrossRef]

171. Su, X.; Matthay, M.A.; Malik, A.B. Requisite role of the cholinergic α7 nicotinic acetylcholine receptor pathway in suppressing Gram-negative sepsis-induced acute lung inflammatory injury. *J. Immunol.* **2010**, *184*, 401–410. [CrossRef] [PubMed]

172. Vivier, E.; Tomasello, E.; Baratin, M.; Walzer, T.; Ugolini, S. Functions of natural killer cells. *Nat. Immunol.* **2008**, *9*, 503–510. [CrossRef] [PubMed]

173. Zanetti, S.R.; Ziblat, A.; Torres, N.I.; Zwirner, N.W.; Bouzat, C. Expression and Functional Role of α7 Nicotinic Receptor in Human Cytokine-stimulated Natural Killer (NK) Cells. *J. Biol. Chem.* **2016**, *291*, 16541–16552. [CrossRef] [PubMed]

174. 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. [CrossRef]

175. Hao, J.; Shi, F.D.; Abdelwahab, M.; Shi, S.X.; Simard, A.; Whiteaker, P.; Lukas, R.; Zhou, Q. Nicotinic receptor β2 determines NK cell-dependent metastasis in a murine model of metastatic lung cancer. *PLoS ONE* **2013**, *8*, e57495. [CrossRef]

176. Jiang, J.L.; Qiu, Y.H.; Peng, Y.P. Effect of acetylcholine on the cytotoxicity of natural killer cells. *Chin. J. Appl. Physiol.* **2005**, *21*, 330–333.

177. Toma, W.; Ulker, E.; Alqasem, M.; AlSharari, S.D.; McIntosh, J.M.; Damaj, M.I. Behavioral and Molecular Basis of Cholinergic Modulation of Pain: Focus on Nicotinic Acetylcholine Receptors. *Curr. Top. Behav. Neurosci.* **2020**, *45*, 153–166.

178. Zhou, Y.Q.; Liu, D.Q.; Liu, C.; Xu, A.J.; Tian, Y.K.; Mei, W.; Tian, X.B. Targeting α7 nicotinic acetylcholine receptors for chronic pain. *Front. Mol. Neurosci.* **2022**, *15*, 970040. [CrossRef]

179. Alfonso-Rodriguez, J.; Wang, S.; Zeng, X.; Candiotti, K.A.; Zhang, Y. Mechanism of Electroacupuncture Analgesia on Nicotine Withdrawal-Induced Hyperalgesia in a Rat Model. *Evid. Based Complement. Altern. Med.* **2022**, *2022*, 7975803. [CrossRef]

180. Papke, R.L.; Horenstein, N.A. Therapeutic Targeting of α7 Nicotinic Acetylcholine Receptors. *Pharm. Rev.* **2021**, *73*, 1118–1149. [CrossRef]

181. Quadri, M.; Bagdas, D.; Toma, W.; Stokes, C.; Horenstein, N.A.; Damaj, M.I.; Papke, R.L. The Antinociceptive and Anti-Inflammatory Properties of the α7 nAChR Weak Partial Agonist p-CF(3) N,N-diethyl-N′-phenylpiperazine. *J. Pharm. Exp. Ther.* **2018**, *367*, 203–214. [CrossRef] [PubMed]

182. Papke, R.L.; Andleeb, H.; Stokes, C.; Quadri, M.; Horenstein, N.A. Selective Agonists and Antagonists of α9 Versus α7 Nicotinic Acetylcholine Receptors. *ACS Chem. Neurosci.* **2022**, *13*, 624–637. [CrossRef]

183. Richter, K.; Papke, R.L.; Stokes, C.; Roy, D.C.; Espinosa, E.S.; Wolf, P.M.K.; Hecker, A.; Liese, J.; Singh, V.K.; Padberg, W.; et al. Comparison of the Anti-inflammatory Properties of Two Nicotinic Acetylcholine Receptor Ligands, Phosphocholine and pCF3-diEPP. *Front. Cell. Neurosci.* **2022**, *16*, 779081. [CrossRef] [PubMed]

184. Di Lascio, S.; Fornasari, D.; Benfante, R. The Human-Restricted Isoform of the α7 nAChR, CHRFAM7A: A Double-Edged Sword in Neurological and Inflammatory Disorders. *Int. J. Mol. Sci.* **2022**, *23*, 3463. [CrossRef]

185. Maroli, A.; Di Lascio, S.; Drufuca, L.; Cardani, S.; Setten, E.; Locati, M.; Fornasari, D.; Benfante, R. Effect of donepezil on the expression and responsiveness to LPS of CHRNA7 and CHRFAM7A in macrophages: A possible link to the cholinergic anti-inflammatory pathway. *J. Neuroimmunol.* **2019**, *332*, 155–166. [CrossRef]

186. de Lucas-Cerrillo, A.M.; Maldifassi, M.C.; Arnalich, F.; Renart, J.; Atienza, G.; Serantes, R.; Cruces, J.; Sanchez-Pacheco, A.; Andres-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. [CrossRef]

187. Wang, Y.; Xiao, C.; Indersmitten, T.; Freedman, R.; Leonard, S.; Lester, H.A. The duplicated α7 subunits assemble and form functional nicotinic receptors with the full-length α7. *J. Biol. Chem.* **2014**, *289*, 26451–26463. [CrossRef]

188. Araud, T.; Graw, S.; Berger, R.; Lee, M.; Neveu, E.; Bertrand, D.; Leonard, S. The chimeric gene CHRFAM7A, a partial duplication of the CHRNA7 gene, is a dominant negative regulator of α7*nAChR function. *Biochem. Pharm.* **2011**, *82*, 904–914. [CrossRef] [PubMed]

189. Martin-Sanchez, C.; Ales, E.; Balseiro-Gomez, S.; Atienza, G.; Arnalich, F.; Bordas, A.; Cedillo, J.L.; Extremera, M.; Chavez-Reyes, A.; Montiel, C. The human-specific duplicated α7 gene inhibits the ancestral α7, negatively regulating nicotinic acetylcholine receptor-mediated transmitter release. *J. Biol. Chem.* **2021**, *296*, 100341. [CrossRef]

190. Peng, W.; Mao, L.; Dang, X. The emergence of the uniquely human α7 nicotinic acetylcholine receptor gene and its roles in inflammation. *Gene* **2022**, *842*, 146777. [CrossRef]

191. Courties, A.; Petit, J.; Do, A.; Legris, M.; Kouki, I.; Pigenet, A.; Sacitharan, P.K.; Ehkirch, F.P.; Berenbaum, F.; Sellam, J. α-7 Nicotinic Receptor Dampens Murine Osteoblastic Response to Inflammation and Age-Related Osteoarthritis. *Front. Immunol.* **2022**, *13*, 842538. [CrossRef] [PubMed]

192. Courties, A.; Olmer, M.; Myers, K.; Ordoukhanian, P.; Head, S.R.; Natarajan, P.; Berenbaum, F.; Sellam, J.; Lotz, M.K. Human-specific duplicate CHRFAM7A gene is associated with more severe osteoarthritis and amplifies pain behaviours. *Ann. Rheum. Dis.* **2023**. [CrossRef] [PubMed]

193. Baird, A.; Coimbra, R.; Dang, X.; Eliceiri, B.P.; Costantini, T.W. Up-regulation of the human-specific CHRFAM7A gene in inflammatory bowel disease. *BBA Clin.* **2016**, *5*, 66–71. [CrossRef] [PubMed]

194. Zhou, B.; Zhang, Y.; Dang, X.; Li, B.; Wang, H.; Gong, S.; Li, S.; Meng, F.; Xing, J.; Li, T.; et al. Up-regulation of the human-specific CHRFAM7A gene protects against renal fibrosis in mice with obstructive nephropathy. *J. Cell. Mol. Med.* **2023**, *27*, 52–65. [CrossRef] [PubMed]

195. Huang, W.; Kabbani, N.; Brannan, T.K.; Lin, M.K.; Theiss, M.M.; Hamilton, J.F.; Ecklund, J.M.; Conley, Y.P.; Vodovotz, Y.; Brienza, D.; et al. Association of a Functional Polymorphism in the CHRFAM7A Gene with Inflammatory Response Mediators and Neuropathic Pain after Spinal Cord Injury. *J. Neurotrauma* **2019**, *36*, 3026–3033. [CrossRef]

196. Hone, A.J.; Servent, D.; McIntosh, J.M. α9-containing nicotinic acetylcholine receptors and the modulation of pain. *Br. J. Pharm.* **2018**, *175*, 1915–1927. [CrossRef] [PubMed]

197. Margiotta, F.; Micheli, L.; Ciampi, C.; Ghelardini, C.; McIntosh, J.M.; Di Cesare Mannelli, L. Conus regius-Derived Conotoxins: Novel Therapeutic Opportunities from a Marine Organism. *Mar. Drugs* **2022**, *20*, 773. [CrossRef]

198. Li, X.; Tae, H.S.; Chu, Y.; Jiang, T.; Adams, D.J.; Yu, R. Medicinal chemistry, pharmacology, and therapeutic potential of α-conotoxins antagonizing the α9α10 nicotinic acetylcholine receptor. *Pharmacol. Ther.* **2021**, *222*, 107792. [CrossRef]

199. Pacini, A.; Micheli, L.; Maresca, M.; Branca, J.J.; McIntosh, J.M.; Ghelardini, C.; Di Cesare Mannelli, L. The α9α10 nicotinic receptor antagonist α-conotoxin RgIA prevents neuropathic pain induced by oxaliplatin treatment. *Exp. Neurol.* **2016**, *282*, 37–48. [CrossRef]

200. Rabik, C.A.; Dolan, M.E. Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat. Rev.* **2007**, *33*, 9–23. [CrossRef]

201. Cragg, G.M. Paclitaxel (Taxol): A success story with valuable lessons for natural product drug discovery and development. *Med. Res. Rev.* **1998**, *18*, 315–331. [CrossRef]

202. Song, S.J.; Min, J.; Suh, S.Y.; Jung, S.H.; Hahn, H.J.; Im, S.A.; Lee, J.Y. Incidence of taxane-induced peripheral neuropathy receiving treatment and prescription patterns in patients with breast cancer. *Support. Care Cancer Off. J. Multinatl. Assoc. Support. Care Cancer* **2017**, *25*, 2241–2248. [CrossRef] [PubMed]

203. Staff, N.P.; Cavaletti, G.; Islam, B.; Lustberg, M.; Psimaras, D.; Tamburin, S. Platinum-induced peripheral neurotoxicity: From pathogenesis to treatment. *J. Peripher. Nerv. Syst. JPNS* **2019**, *24*, S26–S39. [CrossRef] [PubMed]

204. Salat, K. Chemotherapy-induced peripheral neuropathy-part 2: Focus on the prevention of oxaliplatin-induced neurotoxicity. *Pharmacol. Rep. PR* **2020**, *72*, 508–527. [CrossRef] [PubMed]

205. Ma, J.; Kavelaars, A.; Dougherty, P.M.; Heijnen, C.J. Beyond symptomatic relief for chemotherapy-induced peripheral neuropathy: Targeting the source. *Cancer* **2018**, *124*, 2289–2298. [CrossRef]

206. Dyachenko, I.A.; Palikova, Y.A.; Palikov, V.A.; Korolkova, Y.V.; Kazakov, V.A.; Egorova, N.S.; Garifulina, A.I.; Utkin, Y.N.; Tsetlin, V.I.; Kryukova, E.V. α-Conotoxin RgIA and oligoarginine R8 in the mice model alleviate long-term oxaliplatin induced neuropathy. *Biochimie* **2022**, *194*, 127–136. [CrossRef]

207. Li, Z.; Han, X.; Hong, X.; Li, X.; Gao, J.; Zhang, H.; Zheng, A. Lyophilization Serves as an Effective Strategy for Drug Development of the α9α10 Nicotinic Acetylcholine Receptor Antagonist α-Conotoxin GeXIVA[1,2]. *Mar. Drugs* **2021**, *19*, 121. [CrossRef]

208. Di Cesare Mannelli, L.; Cinci, L.; Micheli, L.; Zanardelli, M.; Pacini, A.; McIntosh, J.M.; Ghelardini, C. α-conotoxin RgIA protects against the development of nerve injury-induced chronic pain and prevents both neuronal and glial derangement. *Pain* **2014**, *155*, 1986–1995. [CrossRef]

209. Holtman, J.R.; Dwoskin, L.P.; Dowell, C.; Wala, E.P.; Zhang, Z.; Crooks, P.A.; McIntosh, J.M. The novel small molecule α9α10 nicotinic acetylcholine receptor antagonist ZZ-204G is analgesic. *Eur. J. Pharm.* **2011**, *670*, 500–508. [CrossRef]

210. Westlake, S.; Jones, M.; Sharma, K.D.; Xie, J.Y. Letters to the editor: Nicotinic acetylcholine receptor ligands as potential targets for managing neuropathic pain induced by diabetic peripheral neuropathy. *eNeurologicalSci* **2022**, *28*, 100416. [CrossRef]

211. Lips, K.S.; Pfeil, U.; Kummer, W. Coexpression of α 9 and α 10 nicotinic acetylcholine receptors in rat dorsal root ganglion neurons. *Neuroscience* **2002**, *115*, 1–5. [CrossRef]

212. Shelukhina, I.; Paddenberg, R.; Kummer, W.; Tsetlin, V. Functional expression and axonal transport of α7 nAChRs by peptidergic nociceptors of rat dorsal root ganglion. *Brain Struct. Funct.* **2015**, *220*, 1885–1899. [CrossRef]

213. McIntosh, J.M.; Absalom, N.; Chebib, M.; Elgoyhen, A.B.; Vincler, M. α9 nicotinic acetylcholine receptors and the treatment of pain. *Biochem. Pharm.* **2009**, *78*, 693–702. [CrossRef] [PubMed]

214. Vincler, M.; McIntosh, J.M. Targeting the α9α10 nicotinic acetylcholine receptor to treat severe pain. *Expert Opin. Ther. Targets* **2007**, *11*, 891–897. [CrossRef]

215. Moalem, G.; Tracey, D.J. Immune and inflammatory mechanisms in neuropathic pain. *Brain Res. Rev.* **2006**, *51*, 240–264. [CrossRef]

216. Huynh, P.N.; Christensen, S.B.; McIntosh, J.M. RgIA4 Prevention of Acute Oxaliplatin-Induced Cold Allodynia Requires α9-Containing Nicotinic Acetylcholine Receptors and CD3^{+} T-Cells. *Cells* **2022**, *11*, 3561. [CrossRef] [PubMed]

217. Callaghan, B.; Haythornthwaite, A.; Berecki, G.; Clark, R.J.; Craik, D.J.; Adams, D.J. Analgesic α-conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABAB receptor activation. *J. Neurosci.* **2008**, *28*, 10943–10951. [CrossRef] [PubMed]

218. Cuny, H.; de Faoite, A.; Huynh, T.G.; Yasuda, T.; Berecki, G.; Adams, D.J. γ-Aminobutyric acid type B (GABA$_B$) receptor expression is needed for inhibition of N-type (Ca$_v$2.2) calcium channels by analgesic α-conotoxins. *J. Biol. Chem.* **2012**, *287*, 23948–23957. [CrossRef]

219. Daly, N.L.; Callaghan, B.; Clark, R.J.; Nevin, S.T.; Adams, D.J.; Craik, D.J. Structure and activity of α-conotoxin PeIA at nicotinic acetylcholine receptor subtypes and GABA(B) receptor-coupled N-type calcium channels. *J. Biol. Chem.* **2011**, *286*, 10233–10237. [CrossRef]

220. Huynh, T.G.; Cuny, H.; Slesinger, P.A.; Adams, D.J. Novel mechanism of voltage-gated N-type (Cav2.2) calcium channel inhibition revealed through α-conotoxin Vc1.1 activation of the GABA(B) receptor. *Mol. Pharm.* **2015**, *87*, 240–250. [CrossRef]

221. van Lierop, B.J.; Robinson, S.D.; Kompella, S.N.; Belgi, A.; McArthur, J.R.; Hung, A.; MacRaild, C.A.; Adams, D.J.; Norton, R.S.; Robinson, A.J. Dicarba α-conotoxin Vc1.1 analogues with differential selectivity for nicotinic acetylcholine and GABAB receptors. *ACS Chem. Biol.* **2013**, *8*, 1815–1821. [CrossRef]

222. Callaghan, B.; Adams, D.J. Analgesic α-conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in sensory neurons of α9 nicotinic receptor knockout mice. *Channels* **2010**, *4*, 51–54. [CrossRef]

223. Adams, D.J.; Callaghan, B.; Berecki, G. Analgesic conotoxins: Block and G protein-coupled receptor modulation of N-type (Ca(V) 2.2) calcium channels. *Br. J. Pharm.* **2012**, *166*, 486–500. [CrossRef] [PubMed]

224. Yousuf, A.; Wu, X.; Bony, A.R.; Sadeghi, M.; Huang, Y.; Craik, D.J.; Adams, D.J. αO-Conotoxin GeXIVA isomers modulate N-type calcium (CaV 2.2) channels and inwardly-rectifying potassium (GIRK) channels via GABAB receptor activation. *J. Neurochem.* **2022**, *160*, 154–171. [CrossRef] [PubMed]

225. Deer, T.R.; Pope, J.E.; Hanes, M.C.; McDowell, G.C. Intrathecal Therapy for Chronic Pain: A Review of Morphine and Ziconotide as Firstline Options. *Pain Med.* **2019**, *20*, 784–798. [CrossRef]

226. Wright, A.B.; Norimatsu, Y.; McIntosh, J.M.; Elmslie, K.S. Limited efficacy of α-conopeptides, Vc1.1 and RgIA, to inhibit sensory neuron Ca(V) current. *eNeuro* **2015**, *2*, e0057-14.2015. [CrossRef] [PubMed]

227. Mohammadi, S.; Christie, M.J. α9-nicotinic acetylcholine receptors contribute to the maintenance of chronic mechanical hyperalgesia, but not thermal or mechanical allodynia. *Mol. Pain* **2014**, *10*, 64. [CrossRef]

228. Napier, I.A.; Klimis, H.; Rycroft, B.K.; Jin, A.H.; Alewood, P.F.; Motin, L.; Adams, D.J.; Christie, M.J. Intrathecal α-conotoxins Vc1.1, AuIB and MII acting on distinct nicotinic receptor subtypes reverse signs of neuropathic pain. *Neuropharmacology* **2012**, *62*, 2202–2207. [CrossRef]

229. Zheng, N.; Christensen, S.B.; Dowell, C.; Purushottam, L.; Skalicky, J.J.; McIntosh, J.M.; Chou, D.H. Discovery of Methylene Thioacetal-Incorporated α-RgIA Analogues as Potent and Stable Antagonists of the Human α9α10 Nicotinic Acetylcholine Receptor for the Treatment of Neuropathic Pain. *J. Med. Chem.* **2021**, *64*, 9513–9524. [CrossRef]

230. Zhu, X.; Yuan, M.; Wang, H.; Zhangsun, D.; Yu, G.; Che, J.; Luo, S. Novel αO-conotoxin GeXIVA[1,2] Nonaddictive Analgesic with Pharmacokinetic Modelling-Based Mechanistic Assessment. *Pharmaceutics* **2022**, *14*, 1789. [CrossRef]

231. Jin, A.H.; Muttenthaler, M.; Dutertre, S.; Himaya, S.W.A.; Kaas, Q.; Craik, D.J.; Lewis, R.J.; Alewood, P.F. Conotoxins: Chemistry and Biology. *Chem. Rev.* **2019**, *119*, 11510–11549. [CrossRef] [PubMed]

232. Akondi, K.B.; Muttenthaler, M.; Dutertre, S.; Kaas, Q.; Craik, D.J.; Lewis, R.J.; Alewood, P.F. Discovery, synthesis, and structure-activity relationships of conotoxins. *Chem. Rev.* **2014**, *114*, 5815–5847. [CrossRef]

233. Li, L.; Li, Z.; Guo, Y.; Zhang, K.; Mi, W.; Liu, J. Preparation of uniform-sized GeXIVA[1,2]-loaded PLGA microspheres as long-effective release system with high encapsulation efficiency. *Drug Deliv.* **2022**, *29*, 2283–2295. [CrossRef]

234. Bavo, F.; Pallavicini, M.; Pucci, S.; Appiani, R.; Giraudo, A.; Eaton, B.; Lucero, L.; Gotti, C.; Moretti, M.; Whiteaker, P.; et al. From 2-Triethylammonium Ethyl Ether of 4-Stilbenol (MG624) to Selective Small-Molecule Antagonists of Human α9α10 Nicotinic Receptor by Modifications at the Ammonium Ethyl Residue. *J. Med. Chem.* **2022**, *65*, 10079–10097. [CrossRef]

235. Huang, Q.; Chu, X.; Zhang, H.; Yu, S.; Zhang, L.; Zhang, X.; Yu, R.; Guo, C.; Dai, Q. Discovery and Structural and Functional Characterization of a Novel A-Superfamily Conotoxin Targeting α9α10 Nicotinic Acetylcholine Receptor. *ACS Chem. Biol.* **2022**, *17*, 2483–2494. [CrossRef] [PubMed]

236. Hueffer, K.; Khatri, S.; Rideout, S.; Harris, M.B.; Papke, R.L.; Stokes, C.; Schulte, M.K. Rabies virus modifies host behaviour through a snake-toxin like region of its glycoprotein that inhibits neurotransmitter receptors in the CNS. *Sci. Rep.* **2017**, *7*, 12818. [CrossRef] [PubMed]

237. Embregts, C.W.E.; Begeman, L.; Voesenek, C.J.; Martina, B.E.E.; Koopmans, M.P.G.; Kuiken, T.; GeurtsvanKessel, C.H. Street RABV Induces the Cholinergic Anti-inflammatory Pathway in Human Monocyte-Derived Macrophages by Binding to nAChr α7. *Front. Immunol.* **2021**, *12*, 622516. [CrossRef] [PubMed]

238. Bu, X.; Zhang, A.; Chen, Z.; Zhang, X.; Zhang, R.; Yin, C.; Zhang, J.; Zhang, Y.; Yan, Y. Migration of gastric cancer is suppressed by recombinant Newcastle disease virus (rL-RVG) via regulating α7-nicotinic acetylcholine receptors/ERK- EMT. *BMC Cancer* **2019**, *19*, 976. [CrossRef]

239. Callaway, H.M.; Zyla, D.; Larrous, F.; de Melo, G.D.; Hastie, K.M.; Avalos, R.D.; Agarwal, A.; Corti, D.; Bourhy, H.; Saphire, E.O. Structure of the rabies virus glycoprotein trimer bound to a prefusion-specific neutralizing antibody. *Sci. Adv.* **2022**, *8*, eabp9151. [CrossRef]

240. Neri, P.; Bracci, L.; Rustici, M.; Santucci, A. Sequence homology between HIV gp120, rabies virus glycoprotein, and snake venom neurotoxins. Is the nicotinic acetylcholine receptor an HIV receptor? *Arch. Virol.* **1990**, *114*, 265–269. [CrossRef]

241. Bracci, L.; Lozzi, L.; Rustici, M.; Neri, P. Binding of HIV-1 gp120 to the nicotinic receptor. *FEBS Lett.* **1992**, *311*, 115–118. [CrossRef] [PubMed]

242. Delgado-Vélez, M.; Lasalde-Dominicci, J.A. The Cholinergic Anti-Inflammatory Response and the Role of Macrophages in HIV-Induced Inflammation. *Int. J. Mol. Sci.* **2018**, *19*, 1473. [CrossRef] [PubMed]

243. Capó-Vélez, C.M.; Delgado-Vélez, M.; Báez-Pagán, C.A.; Lasalde-Dominicci, J.A. Nicotinic Acetylcholine Receptors in HIV: Possible Roles During HAND and Inflammation. *Cell. Mol. Neurobiol.* **2018**, *38*, 1335–1348. [CrossRef]

244. Farsalinos, K.; Barbouni, A.; Niaura, R. Systematic review of the prevalence of current smoking among hospitalized COVID-19 patients in China: Could nicotine be a therapeutic option? *Intern. Emerg. Med.* **2020**, *15*, 845–852. [CrossRef]

245. Fudim, M.; Qadri, Y.J.; Ghadimi, K.; MacLeod, D.B.; Molinger, J.; Piccini, J.P.; Whittle, J.; Wischmeyer, P.E.; Patel, M.R.; Ulloa, L. Implications for Neuromodulation Therapy to Control Inflammation and Related Organ Dysfunction in COVID-19. *J. Cardiovasc. Transl. Res.* **2020**, *13*, 894–899. [CrossRef] [PubMed]

246. Kloc, M.; Ghobrial, R.M.; Kubiak, J.Z. How nicotine can inhibit cytokine storm in the lungs and prevent or lessen the severity of COVID-19 infection? *Immunol. Lett.* **2020**, *224*, 28–29. [CrossRef] [PubMed]

247. Lupacchini, L.; Maggi, F.; Tomino, C.; De Dominicis, C.; Mollinari, C.; Fini, M.; Bonassi, S.; Merlo, D.; Russo, P. Nicotine Changes Airway Epithelial Phenotype and May Increase the SARS-CoV-2 Infection Severity. *Molecules* **2020**, *26*, 101. [CrossRef]

248. Benowitz, N.L.; Goniewicz, M.L.; Halpern-Felsher, B.; Krishnan-Sarin, S.; Ling, P.M.; O'Connor, R.J.; Pentz, M.A.; Robertson, R.M.; Bhatnagar, A. Tobacco product use and the risks of SARS-CoV-2 infection and COVID-19: Current understanding and recommendations for future research. *Lancet Respir. Med.* **2022**, *10*, 900–915. [CrossRef]

249. Clift, A.K.; von Ende, A.; Tan, P.S.; Sallis, H.M.; Lindson, N.; Coupland, C.A.C.; Munafò, M.R.; Aveyard, P.; Hippisley-Cox, J.; Hopewell, J.C. Smoking and COVID-19 outcomes: An observational and Mendelian randomisation study using the UK Biobank cohort. *Thorax* **2022**, *77*, 65–73. [CrossRef]

250. Maggi, F.; Rosellini, A.; Spezia, P.G.; Focosi, D.; Macera, L.; Lai, M.; Pistello, M.; de Iure, A.; Tomino, C.; Bonassi, S.; et al. Nicotine upregulates ACE2 expression and increases competence for SARS-CoV-2 in human pneumocytes. *ERJ Open Res.* **2021**, *7*, 00713–02020. [CrossRef]

251. Russo, P.; Bonassi, S.; Giacconi, R.; Malavolta, M.; Tomino, C.; Maggi, F. COVID-19 and smoking: Is nicotine the hidden link? *Eur. Respir. J.* **2020**, *55*, 2001116. [CrossRef] [PubMed]

252. Allahverdi Khani, M.; SalehiRad, M.; Darbeheshti, S.; Motaghinejad, M. Survival of COVID-19 patients requires precise immune regulation: The hypothetical immunoprotective role of nicotinic agonists. *Med. Hypotheses* **2020**, *143*, 109871. [CrossRef] [PubMed]

253. Ballestero, J.A.; Plazas, P.V.; Kracun, S.; Gomez-Casati, M.E.; Taranda, J.; Rothlin, C.V.; Katz, E.; Millar, N.S.; Elgoyhen, A.B. Effects of quinine, quinidine, and chloroquine on α9α10 nicotinic cholinergic receptors. *Mol. Pharm.* **2005**, *68*, 822–829. [CrossRef] [PubMed]

254. Changeux, J.P.; Amoura, Z.; Rey, F.A.; Miyara, M. A nicotinic hypothesis for COVID-19 with preventive and therapeutic implications. *Comptes Rendus Biol.* **2020**, *343*, 33–39. [CrossRef]

255. Farsalinos, K.; Eliopoulos, E.; Leonidas, D.D.; Papadopoulos, G.E.; Tzartos, S.; Poulas, K. Nicotinic Cholinergic System and COVID-19: In Silico Identification of an Interaction between SARS-CoV-2 and Nicotinic Receptors with Potential Therapeutic Targeting Implications. *Int. J. Mol. Sci.* **2020**, *21*, 5807. [CrossRef]

256. Lagoumintzis, G.; Chasapis, C.T.; Alexandris, N.; Kouretas, D.; Tzartos, S.; Eliopoulos, E.; Farsalinos, K.; Poulas, K. Nicotinic cholinergic system and COVID-19: In silico identification of interactions between α7 nicotinic acetylcholine receptor and the cryptic epitopes of SARS-Co-V and SARS-CoV-2 Spike glycoproteins. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **2021**, *149*, 112009. [CrossRef]

257. Oliveira, A.S.F.; Ibarra, A.A.; Bermudez, I.; Casalino, L.; Gaieb, Z.; Shoemark, D.K.; Gallagher, T.; Sessions, R.B.; Amaro, R.E.; Mulholland, A.J. A potential interaction between the SARS-CoV-2 spike protein and nicotinic acetylcholine receptors. *Biophys. J.* **2021**, *120*, 983–993. [CrossRef]

258. Lykhmus, O.; Kalashnyk, O.; Koval, L.; Krynina, O.; Komisarenko, S.; Skok, M. Immunization with 674-685 fragment of SARS-CoV-2 spike protein induces neuroinflammation and impairs episodic memory of mice. *Biochem. Biophys. Res. Commun.* **2022**, *622*, 57–63. [CrossRef]

259. Chrestia, J.F.; Oliveira, A.S.; Mulholland, A.J.; Gallagher, T.; Bermudez, I.; Bouzat, C. A Functional Interaction Between Y674-R685 Region of the SARS-CoV-2 Spike Protein and the Human α7 Nicotinic Receptor. *Mol. Neurobiol.* **2022**, *59*, 6076–6090. [CrossRef]

260. Godellas, N.E.; Cymes, G.D.; Grosman, C. An experimental test of the nicotinic hypothesis of COVID-19. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2204242119. [CrossRef]