Targeting the α7 nicotinic acetylcholine receptor—A novel road towards the future treatment of skin diseases

Agatha Stegemann | Markus Böhm

Dept. of Dermatology, University of Münster, Germany

Correspondence
Markus Böhm, MD, Associate Professor, Department of Dermatology, Laboratory for Neuroendocrinology of the Skin and Interdisciplinary Endocrinology, University of Münster, Von-Esmarch-Str. 58, 48149 Münster, Germany.
Email: bohmm@uni-muenster.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: STE 2312/1-3

Abstract
Nicotinic acetylcholine receptors (nAChRs) are members of the superfamily of neurotransmitter-gated ion channels. The natural ligand for nAChRs is the endogenous neurotransmitter acetylcholine. Among the nAChRs is the α7nAChR. It is not only expressed by neural tissues but also in the skin. A number of different resident cutaneous cell types including epidermal keratinocytes, sebocytes and dermal fibroblasts express functional α7nAChR. Moreover, cells of the immune system such as lymphocytes, macrophages and monocytes, playing an important role in skin homeostasis, also express α7nAChR. Translational research focusing on the exploitation of the α7nAChR in dermatology has revealed that this neuroendocrine receptor could be promising target for the treatment of inflammatory skin diseases. For example, α7nAChR agonists can counteract transforming growth factor-β1-mediated responses in dermal fibroblasts, key effector cells in scleroderma. In accordance with this α7nAChR, agonists are effective in both inflammation and non-inflammation-driven models of experimentally induced skin fibrosis. Moreover, α7nAChR agonists can modulate expression of proinflammatory cytokines in epidermal keratinocytes that are crucially involved in the pathogenesis of psoriasis and other inflammatory skin diseases. Finally, the capability of α7nAChR agonists to suppress ultraviolet light A/B-induced responses, for example production of proinflammatory cytokines and oxidative stress, the latter crucially involved in dermal photoageing, points to a potential of such agents in the prevention of extrinsic skin ageing. Therefore, emphasis on translational research targeting the α7nAChR in skin may lead to the development of new treatment and prevention modalities against fibrosclerotic skin diseases, psoriasis vulgaris, atopic dermatitis, acne, photodermatoses and extrinsic skin ageing.

KEYWORDS
dermatology, inflammation, scleroderma, skin diseases, α7nAChR
1 | BASIC BIOCHEMISTRY AND BIOLOGY OF THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR

Nicotinic acetylcholine receptors (nAChRs) are members of the superfamily of neurotransmitter-gated ion channels. The endogenous neurotransmitter acetylcholine and nicotine, one of the most common pollutants, bind to these receptors.\(^1\) The structure of these receptors is formed by five protein subunits (α,β,γ,δ,ε) which are able to interact with each other to form complexes. Not less than 10 different α and 4 different β subunits and thus, a large number of different receptor complexes with different binding affinities, exist. The nAChRs are distributed all over the central nervous system as well as the peripheral tissues.\(^2,3\) Ligand binding by the ion channel leads to an ion influx resulting in activation of ion-dependent signal transduction cascades.

The α7nAChR is one of the most abundant nAChRs within the brain. It consists of five identical α7 subunits and has a higher Ca\(^{2+}\) permeability than other nAChRs with a permeability ratio of Ca\(^{2+}\)/Na\(^{+}\) of 5.9.\(^4,5\) The α7nAChR subunits can form homopentamers as well heteropentamers. However, no data exist about the expression of α7nAChR heteropentamers in skin. Although α7nAChR agonists typically signal via intracellular levels of Ca\(^{2+}\) other canonical signalling pathways, in part cell-type-specific, for example, the signal transducer and activator of transcription (STAT) pathway and nuclear factor kappa B (NFκB) signalling can be activated after Ca\(^{2+}\) entry.\(^6-10\) In addition, α7nAChR-mediated signalling can modulate several other signalling pathways including extracellular signal-regulated kinases (ERK 44/42)\(^7\) and prostaglandin E\(_2\)/protein kinase A.\(^11,12\)

The α7nAChR was originally described as a neuronal receptor in the central nervous system. Neuronal nAChRs have been extensively characterized regarding their biological role in peripheral and central neurons revealing that nAChRs contribute to the control of resting membrane potential, modulation of synaptic transmission and mediation of fast excitatory transmission.\(^13\) nAChRs have been demonstrated to be involved in cognitive processes such as learning, memory and control of movement whereas dysfunction of nAChR has been linked to human diseases such as schizophrenia, Alzheimer’s and Parkinson’s diseases.\(^14\) In addition to neurons in the brain, several non-neuronal tissues including cells of the immune system such as lymphocytes, macrophages and dendritic cells express α7nAChRs.\(^15\) It has been shown that the α7nAChR is crucial for the cholinergic anti-inflammatory action as α7nAChR-deficient animals do not respond anymore with inhibition of synthesis of tumor necrosis factor-α to stimulation of the vagus nerve.\(^16\) Therefore, targeting the α7nAChR has emerged as an important anti-inflammatory pathway that controls neurogenic inflammation. However, the fact that the α7nAChR is expressed also by cells of the immune system as well as by non-neuronal cells of various tissues supports the concept that specific α7nAChR agonists can be exploited also for the treatment of a diversity of inflammatory diseases outside the neural context. Indeed, acetylcholine due its non-selectivity towards the α7nAChR can hardly be used therapeutically for such indications. However, both agonistic and antagonistic ligands have been designed and characterized pharmacaceutically in the last years. Specific and highly selective α7nAChR agonists include AR-R17779, PHA-543613 and PNU-282987, whereas α-bungarotoxin (α-BTX) and methyllycaconitine are α7nAChR antagonists.\(^17,18\) A list of α7nAChR ligands with their properties is shown in Table 1.\(^39-41\) Although α-BTX can also bind α1, α9 and α10 nAChR subunits, the most relevant subunit in human dermal fibroblasts is the α7nAChR.\(^42\) Interestingly, the α7nAChR agonist AR-R17779 was described to attenuate collagen-induced arthritis in mice.\(^43\) Another agonist GTS-21 improved survival in murine endotoxaemia and sepsis and attenuated inflammation and injury during experimental pancreatitis.\(^23,43\) However, despite their use in vitro as well as in preclinical animal model inflammatory diseases, none of these agents has been shown to have the light of the day in dermatological routine yet. Interestingly, tropisetron, an approved antiemetic and classical serotonin receptor antagonist possesses molecular structure homology, also binds to the α7nAChR and acts here as a partial agonist.\(^44,45\) Anecdotal reports exist that tropisetron can elicit some modulatory effects in distinct rheumatic diseases including those of the skin\(^46\) and an observation that was elucidated in detail by our mechanistic in vitro and in vivo studies.\(^39\) For example, in patients with progressive systemic sclerosis an improvement of some symptoms was reported in a case report and tropisetron was shown to have beneficial in fibromyalgia.\(^46,47\)

2 | EXPRESSION, DISTRIBUTION AND REGULATION OF α7nAChR IN SKIN

Although originally described as a neuronal receptor, the α7nAChR is also expressed in a number of non-neuronal tissues including skin. Regarding cutaneous expression, immunoreactivity for the α7nAChR was initially detected in normal human skin within the interfollicular epidermis, the hair follicle, sebaceous gland and in endothelial and fibroblastic cells. Especially differentiated sebocytes as well as myoepithelial cells were highly positive.\(^42\) Subsequent studies employing detection methods other than immunofluorescence analysis revealed expression of α7nAChR in human epidermal keratinocytes\(^48,49\) and dermal human fibroblasts cultured in vitro.\(^50\) Our own group confirmed the presence of functional α7nAChRs in human dermal fibroblasts which reacted to agonistic stimulation with Ca\(^{2+}\) influx.\(^39\) Whereas melanocytes seem to lack expression of the α7nAChR, primary human sebocytes in vitro as well as sebaceous glands in human skin were reported to express α7nAChR.\(^51\) Besides the cutaneous system, α7nAChR is also expressed in non-cutaneous cell types such as epithelial cells of blood vessels, and immune cells such as lymphocytes and macrophages.\(^52\) α7nAChR activity in skin can be regulated by secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related protein-1 (SLURP-1), a protein exhibiting structure similarity to α-bungarotoxin.\(^53\) SLURP-1 acts as a positive allosteric modulator of α7nAChR was reported to reduce tumor necrosis factor (TNF)-α release by macrophages during wound healing.\(^54\) Another α7nAChR modulator SLURP-2 was found to promote the proliferation of human oral keratinocytes.
via interactions with α3β2nAChRs, while it inhibited cell growth via α7nAChRs. SLURP-2 also significantly enhances acetylcholine-evoked currents.\(^55\) Acetylcholine leads to increased lipid synthesis in a dose-dependent manner in these primary human sebocytes and α-BTX neutralized the upregulatory effect of acetylcholine in these cells.\(^51\) Interestingly, sebum production could be reduced by botulinum toxin (Botox),\(^51\) a natural neurotoxin which prevents acetylcholine release. It is widely used for cosmetic rejuvenation.\(^56\)

### 3 | α7nAChR AS A NOVEL TARGET FOR THE TREATMENT OF SKIN DISEASES

The skin comprises resident as well as transiently residing cells of the non-neuronal cholinergic system like cells of the immune system. Thus, the non-neuronal cholinergic system controls highly complex processes within the skin such as proliferation, differentiation, apoptosis, adhesion and migration of various cell types.

### 3.1 | Scleroderma and other fibrosclerotic skin diseases

Scleroderma is a connective tissue disease characterized by vascular damage, autoimmune phenomena involving both the humoral and cellular immune system, and excessive production of collagen and other components of the extracellular matrix (ECM).\(^57,58\) It can be limited to skin as in morphea but can also involve inner organs such as lung, heart and liver as in systemic sclerosis (SSc). Some years ago, an anecdotal clinical observation prompted us to shed light into the beneficial effects of tropisetron in scleroderma.\(^46\) Albeit approved as a classical serotonin antagonist, this agent has also off-target agonistic effects on α7nAChR as mentioned above. In fact, tropisetron suppressed dose-dependently transforming growth factor (TGF)-β1-induced collagen and α-smooth muscle actin synthesis in human dermal fibroblasts (HDFs).\(^39\) This effect was mediated by activation of the α7nAChR as demonstrated by genetic knock-down of this receptor and pharmacological studies with the α7nAChR antagonist α-BTX. Indeed, tropisetron at 10 µg/ml elicited

| Ligand            | Property                   | Function/Usage                                                                 | References |
|-------------------|----------------------------|-------------------------------------------------------------------------------|------------|
| α-bungarotoxin    | Selective antagonist       | Blocks effects of α7nAChR                                                     | [19,20]    |
| Methyllycaconitine| Selective antagonist       | Neurological disorders                                                        | [21,22]    |
|                   |                            | Cannabis dependence                                                           |            |
| Mecamylamine      | Non-selective antagonist   | Blocks effects of nAChRs                                                       | [23,24]    |
|                   |                            | Antihypertensive                                                              |            |
|                   |                            | Neuropsychiatric disorders                                                    |            |
| Acetylcholine     | Non-selective agonist      | Neurotransmitter                                                               | [25]       |
|                   |                            | Neuromodulator                                                                |            |
| Nicotine          | Non-selective agonist      | Anti-inflammatory                                                             | [9,19,20,26] |
|                   |                            | Antihypertensive                                                              |            |
| Cotinine          | Non-selective agonist      | Nootropic, Antipsychotic                                                      | [27,28]    |
|                   |                            | Antidepressant                                                                |            |
| Anabaseine        | Non-selective agonist      | Release of dopamine and norepinephrine                                         | [29]       |
| PNU-282987        | Selective agonist          | Nootropic                                                                     | [30,31]    |
|                   |                            | Schizophrenia                                                                 |            |
| AR-R17779         | Selective agonist          | Nootropic                                                                     | [32-34]    |
|                   |                            | Anti-inflammatory                                                             |            |
|                   |                            | Antifibrotic                                                                  |            |
| TC-7020           | Selective agonist          | Schizophrenia                                                                 | [35]       |
| CAP55             | Selective agonist          | Anti-inflammatory                                                             | [9]        |
| GTS-21            | Partial agonist            | Alzheimer’s disease                                                           | [23,36]    |
|                   |                            | Schizophrenia                                                                 |            |
|                   |                            | Anti-inflammatory                                                             |            |
| PHA-568487        | Selective agonist          | Schizophrenia                                                                 | [37]       |
| PHA-543613        | Selective agonist          | Schizophrenia                                                                 | [18,34]    |
|                   |                            | Anti-inflammatory                                                             |            |
|                   |                            | Antifibrotic                                                                  |            |
| DMAB              | Partial agonist            | Antinociceptive                                                               | [38]       |
| Tropisetron       | Partial agonist            | Antifibrotic                                                                  | [39-42]    |
|                   |                            | Anti-inflammatory                                                             |            |
|                   |                            | Antioxidative                                                                 |            |

### Table 1 | α7nAChR ligands and their properties

| Ligand            | Property                   | Function/Usage                                                                 | References |
|-------------------|----------------------------|-------------------------------------------------------------------------------|------------|
| α-bungarotoxin    | Selective antagonist       | Blocks effects of α7nAChR                                                     | [19,20]    |
| Methyllycaconitine| Selective antagonist       | Neurological disorders                                                        | [21,22]    |
|                   |                            | Cannabis dependence                                                           |            |
| Mecamylamine      | Non-selective antagonist   | Blocks effects of nAChRs                                                       | [23,24]    |
|                   |                            | Antihypertensive                                                              |            |
|                   |                            | Neuropsychiatric disorders                                                    |            |
| Acetylcholine     | Non-selective agonist      | Neurotransmitter                                                               | [25]       |
|                   |                            | Neuromodulator                                                                |            |
| Nicotine          | Non-selective agonist      | Anti-inflammatory                                                             | [9,19,20,26] |
|                   |                            | Antihypertensive                                                              |            |
| Cotinine          | Non-selective agonist      | Nootropic, Antipsychotic                                                      | [27,28]    |
|                   |                            | Antidepressant                                                                |            |
| Anabaseine        | Non-selective agonist      | Release of dopamine and norepinephrine                                         | [29]       |
| PNU-282987        | Selective agonist          | Nootropic                                                                     | [30,31]    |
|                   |                            | Schizophrenia                                                                 |            |
| AR-R17779         | Selective agonist          | Nootropic                                                                     | [32-34]    |
|                   |                            | Anti-inflammatory                                                             |            |
|                   |                            | Antifibrotic                                                                  |            |
| TC-7020           | Selective agonist          | Schizophrenia                                                                 | [35]       |
| CAP55             | Selective agonist          | Anti-inflammatory                                                             | [9]        |
| GTS-21            | Partial agonist            | Alzheimer’s disease                                                           | [23,36]    |
|                   |                            | Schizophrenia                                                                 |            |
|                   |                            | Anti-inflammatory                                                             |            |
| PHA-568487        | Selective agonist          | Schizophrenia                                                                 | [37]       |
| PHA-543613        | Selective agonist          | Schizophrenia                                                                 | [18,34]    |
|                   |                            | Anti-inflammatory                                                             |            |
|                   |                            | Antifibrotic                                                                  |            |
| DMAB              | Partial agonist            | Antinociceptive                                                               | [38]       |
| Tropisetron       | Partial agonist            | Antifibrotic                                                                  | [39-42]    |
|                   |                            | Anti-inflammatory                                                             |            |
|                   |                            | Antioxidative                                                                 |            |
canonical Ca^{2+} signalling indicating a functional α7nAChR. Notably, α7nAChR was also detected in total skin and dermal fibroblasts established from patients with SSc. At present, we do not know whether expression of α7nAChR or α7nAChR-mediated intracellular signalling is downregulated in SSc. Downregulation of α7nAChR-mediated fibroblast signalling in SSc would indicate a pathogenetic role of this neuroendocrine receptor in SSc. However, targeting the α7nAChR with tropisetron in vivo in wild-type mice revealed that this drug cannot only prevent (simultaneous subcutaneous injections of tropisetron 10 mg/ml and bleomycin 10 µg over 21 days) but also revert an established experimentally induced skin fibrosis (belated subcutaneous injections of tropisetron over 14 days after 21 days of bleomycin treatment) in the bleomycin mouse model of SSc. Subsequent studies from our group could recently demonstrate that a pharmacologically characterized full α7nAChR agonist such as AR-17779 (50 µM) likewise suppresses TGF-β1-induced collagen synthesis in HDFs. Mechanistically, JunB, a member of the activator protein-1 (AP-1) family, was modulated by the α7nAChR agonists AR-17779 at 50 µM and PHA-543613 at 10 µM in HDFs and found to be the mediator of the suppressive effect of α7nAChR activation on TGF-β1-mediated induction of ECM genes. Modulation of this redox-sensitive transcription factor was related to regulation of the respiratory state in mitochondria of HDFs. The higher ROS accumulation induced by TGF-β1 treatment was antagonized by an antioxidative effect of both α7nAChR agonists on the oxidative phosphorylation system (OXPHOS) in HDFs. In vivo, PHA-543613 was both antifibrogenic and antifibrotic in the bleomycin mouse model of SSc. Interestingly, agonistic stimulation of α7nAChR with PHA-543613 and AR-17779 attenuated experimental skin fibrosis in the non-inflammation-driven AdTBRIα7 mouse model indicating fibroblast-mediated and not only anti-inflammatory effects of such agents. In this mouse model, constitutive TGF-β1 receptor activation is induced by adenoviral injections.

These findings are in accordance with data from others who recently reported on a protective role of the α7nAChR in lung and liver fibrosis using α7nAChR agonists as antifibrotic agents. Therefore, these findings are highly encouraging to assess the clinical potential of full α7nAChR agonists in clinical studies of patients with SSc and related diseases, for example morphea or sclerodema, which are still big therapeutic challenges in dermatology. Studies with animals lacking the α7nAChR are currently performed in our laboratory and will further complete our knowledge about the role of this receptor in fibrosis.

### 3.2 Psoriasis vulgaris

Psoriasis is one of the most common immune-mediated skin disorders. Proinflammatory cytokines of the innate immune system, for example TNF, and those of the adaptive immune system, for example interleukin (IL)-17 and IL-23, have emerged as crucial pathogenetic players and new powerful treatments. While expression of α7nAChR has been detected in epidermal keratinocytes in vitro and in situ, nothing is known on the regulation of this receptor in psoriasis. As psoriasis is a hyperproliferative inflammatory skin disease, therapeutic control of keratinocyte proliferation/differentiation is a therapeutic need. In this context, it was shown that the α7nAChR controls homeostasis and terminal differentiation of epidermal keratinocytes required for formation of the skin barrier. Inhibition of the α7nAChR pathway favoured cell cycle progression. These findings point to a possible autoimmune relevance of αnAChR receptors in keratinocyte proliferation/differentiation. Expression of α7nAChR was detected in perivascular regions in synovial tissue of patients with psoriatic arthritis. The cellular localization identified macrophages, fibroblasts, endothelial-like cells as well as T cells and B cells as α7nAChR expressing structures. Acetylcholine levels in skin tissue of patients suffering from psoriasis were significantly higher than in controls. The high expression of α7nAChRs in patients with longer disease durations might represent an attempt by the body to regulate the inflammatory cascade in psoriatic lesions. Interestingly, in psoriasis SLURP-2 expression at RNA level was found to be elevated in lesional skin of psoriasis patients in comparison to non-lesional skin and healthy individuals. However, SLURP-2 preferentially binds to the α3nAChR but also can bind to α7nAChR like SLURP-1. Of note, epidermal keratinocytes are able to synthesize, secrete and degrade acetylcholine. However to the best of our knowledge, it is unknown as to whether in psoriasis secretion of this neuroendocrine mediator by keratinocytes is altered.

Recently, we found that tropisetron in accordance with its effects on dermal fibroblasts suppressed the TNF-α-mediated inflammatory responses of primary human epidermal keratinocytes. As mentioned above neutralizing TNF-α-mediated effects is one of the most powerful therapeutic strategies to treat psoriasis. TNF-α-induced expression of both IL-6 and IL-8 at RNA and protein level was dose-dependently suppressed by tropisetron. Like in dermal fibroblasts, these effects were not mediated via serotonin (5-HT) receptors, the bona fide target receptor for tropisetron, but via α7nAChR. Primary human epidermal keratinocytes did neither express the 5-HT3 receptor nor the 5-HT4 receptor that bind tropisetron. α-BTX, however, neutralized the suppressive effect of tropisetron on TNF-α-mediated cytokine release by human epidermal keratinocytes. Moreover, the full α7nAChR agonist AR-17779 mimicked the counterregulatory effect of tropisetron against TNF-α in these cells. These findings clearly show functional α7nAChRs in human epidermal keratinocytes that mediate anti-inflammatory signalling and actions in epidermal keratinocytes. Based on these promising results, it will be fascinating to explore the in vivo efficacy of full α7nAChR agonists in appropriate in vivo models of psoriasis. Preliminary findings on mice with imiquimod-induced psoriasis-like skin lesions in fact suggest that targeting the α7nAChR by tropisetron has beneficial effects including in vivo suppression of proinflammatory key cytokines of psoriasis.

### 3.3 Atopic dermatitis

Like psoriasis vulgaris, atopic dermatitis is a very common pruritic inflammatory skin disorder with a prevalence of more than 1% in Western countries. Immunopathogenetically, patients with this disease display
an alteration of the Th1/Th2 balance resulting in increased expression levels of various proallergic cytokines including IL-4, IL-5 and IL-13, key players in immunoglobulin E synthesis and eosinophil activation in these patients.\[70,71\] Targeting of some of these proallergic cytokines, especially IL-4 and IL-13, has emerged as a highly effective therapy in atopic dermatitis. However, inhibition of calcineurin, an intracellular phosphatase orchestrating the transcriptional expression of IL-2, IL-12 and IL-23 by lymphocytes, still represents a powerful treatment strategy in both patients with atopic dermatitis and psoriasis vulgaris. Immune cells including T lymphocytes\[72\] and human and murine macrophages do express α7nAChRs.\[16,73\] Interestingly, tropisetron in vitro suppressed cell activation of human T lymphocytes by inhibition of IL-2 synthesis in stimulated T cells. In Jurkat T cells, tropisetron suppressed DNA binding and transcriptional activity of NF-AT and AP-1, presumably by targeting calcineurin.\[45\] In addition, NF-κB activation by distinct stimuli was also suppressed. However, this study lacks evidence that tropisetron elicits its effects via the α7nAChR, although the authors propose a serotonin-independent mode of action. It would be interesting to learn whether full agonists of α7nAChRs have similar effects in these cells. Moreover, testing α7nAChR agonists in murine or rat models of atopic dermatitis, for example in spontaneous atopic dermatitis models such as NC/Nga mice or Flaky tail mice or atopic dermatitis models induced by oxazolone or dinitrofluorobenzene,\[74\] will further extend the so far still preliminary in vitro data on the effect of α7nAChR-mediated signalling in this context of this disease. With regard to the pathogenesis of atopic dermatitis, it will also be worth to investigate expression and α7nAChR-mediated signalling in skin and immune cells compared to healthy individuals. It is known for some time that acetylcholine and choline acetyltransferase expression levels are increased in skin cells of patients with AD where the amount of both was determined in skin biopsies. The increase in acetylcholine levels was more accentuated in lesional skin but still higher in non-lesional areas compared to healthy controls. Moreover, acetyltransferase immunoreactivity was found in keratinocytes, eccrine sweat glands, sebaceous glands and hair papilla.\[75\]

However, acetylcholine is a non-selective agonist to many AChR subtypes such as ionotropic and metabotropic receptors, the latter indirectly linked with ion channels on the plasma membrane, and thus the relative contribution of elevated acetylcholine levels to α7nAChR-mediated signalling remains unknown. Nevertheless, it was shown that lesional atopic dermatitis skin lacks the expression of α7nAChR and expression of its ligand SLURP-1 was low in non-lesional skin. After stress, SLURP-1 and 2 decreased in healthy tissue but in contrast strongly increased in lesional atopic dermatitis skin.\[76\] Reduced non-neuronal cholinergic system markers and α7nAChR-mediated anti-inflammatory signalling in lesional atopic dermatitis skin may propagate cutaneous inflammation. Interestingly, the α7nAChR also has a central role in keeping the homeostasis and regulating terminal differentiation of the epidermis.\[48\] Moreover, defects in epidermal barrier function in stress-induced dermatoses can be improved by nAChR agonists and antagonists via regulation of cornified envelope proteins to maintain barrier permeability and homeostasis.\[77\]

### 3.4 Ultraviolet light-induced skin ageing and photodermatoses

Ultraviolet (UV) light within the natural solar spectrum is the most ubiquitous stressor to human skin. While UVB is mostly absorbed within the epidermis, UVA deeply penetrates into the dermis and induces photoageing.\[78,79\] A pivotal molecular mechanism in the latter is generation of reactive oxygen species (ROS) which is released by dermal fibroblasts resulting in induction of collagen-degrading matrix metalloproteinases (MMPs).\[78,79\] ROS-mediated effects are implicated in the pathogenesis of a variety of photodermatoses like erythropoietic protoporphyria.\[80\] Therefore, we tested whether tropisetron as a partial α7nAChR agonist or AR-R17779 can reduce UVA-induced generation of ROS in HDFs in vitro. Both of these agents dose-dependently reduced generation of H2O2 extracellularly and intracellularly when preincubated for 24 hours while addition of the compounds just before UVA exposure did not have any effect. The latter observation pointed towards in indirect antioxidative effect of α7nAChR agonists. In fact, tropisetron induced catalase expression in HDFs in a time-dependent fashion and genetic knock-down of this antioxidative enzyme significantly reduced the effect of tropisetron on UVA-induced generation of intracellular ROS. Importantly, expression of MMP1 and MMP3 by UVA was strongly suppressed by tropisetron indicating that expression of the final executers of collagen-degradation in dermal photoageing is modulated by α7nAChR agonists.\[40\] Could α7nAChR agonists also work against UVB-mediated effects, for example induction of proinflammatory cytokines and mediators? Interestingly, it was shown some time ago that mice with global genetic ablation of α7nAChR display increased production of IL-1β and IL-6 measured in skin biopsies at mRNA and protein level.\[81\] Interestingly, tropisetron also suppressed UVB-induced induction of TNF-α in human epidermal keratinocytes (our own unpublished data). Here, epidermal keratinocytes were exposed to the physiological dose of UVB at 10 mJ/cm² alone or UVB plus tropisetron at 10 ng/ml followed by measurement of the inflammatory response, for example IL-6 and IL-8 expression. These data indicate that α7nAChR-mediated pathways cannot only interfere with UVA-mediated ROS and MMP induction but also with UVB-mediated skin responses. A summary of the major findings is presented in Table 2\[23-81\].

### 4 OPEN QUESTIONS AND FUTURE PERSPECTIVES

The published scientific literature as presented in this viewpoint essay indicates a promising spectrum of antifibrogenic, anti-biologic, anti-inflammatory and antioxidative effects of α7nAChR agonists with regard to the skin (Figure 1). Most compelling evidence comes from the effects of α7nAChR agonists on fibroblast activation in vitro as well their antifibrogenic and antibiologic effects in vivo in well-established skin fibrosis models. Indeed, there are additional animal models of scleroderma and keloid, for example HOCl-induced fibrosis, TGF-β-transgenic mouse model, graft-versus-host disease model...
**TABLE 2** Overview of the major findings related to α7nAChR

| Model | Intervention | Effect | Reference |
|-------|--------------|--------|-----------|
| Mouse model of SSC | Tropisetron, PHA-543613 | Antifibrotic, Anti-inflammatory | [34,39] |
| Human dermal fibroblasts | Tropisetron, AR-R17779, PHA-543613 | Antifibrotic, Anticollagenic, Antioxidative | [34,39] |
| Mouse model of arthritis | AR-R17779 | Anti-inflammatory | [33] |
| Mouse model of pancreatitis | GTS-21 | Anti-inflammatory | [23,44] |
| AdTBRα1KO mouse model of fibrosis | PHA-5436513, AR-R17779 | Antifibrotic | [34] |
| Mouse model of lung injury | GTS-21 | Anti-inflammatory | [60] |
| Mouse model of liver fibrosis | α7nAChR KO animals | Proinflammatory | [61] |
| Human primary epidermal keratinocytes | Tropisetron, AR-R17779 | Anti-inflammatory | [41] |
| Mouse model of psoriasis | Tropisetron | Anti-inflammatory | [70] |
| T lymphocytes and T cells | Tropisetron | Anti-inflammatory | [42] |
| Human dermal fibroblasts | Tropisetron, AR-R17779 | Antioxidative | [40] |
| Mouse model of UV-induced inflammation | α7nAChR KO animals | Proinflammatory | [82] |

**FIGURE 1** Effects of the α7nAChR and its ligands on human skin cells including dermal fibroblasts, keratinocytes and immune cells. Activation of the α7nAChR has antifibrotic and anti-inflammatory properties, for example it reduces TGF-β1-induced ECM synthesis and UVA-mediated ROS accumulation in HDFs, attenuates IL-6/8 and TNF-α expression in keratinocytes and inhibits the proinflammatory response of T lymphocytes and macrophages. This may lead to biological effects in vivo, for example suppression of skin fibrosis, inflammation and ageing.
and TSK1/2 + mice or rabbit ear model for keloid scarring, which also can be tested. More relevant and informative for the human system, however, would be proof-of-principle clinical trials with either tropisetron as an already approved antiemetic with a partial agonistic α7nAChR effect or with full α7nAChR agonists. With regard to keratinocyte biology, more in vivo data are now urgently needed to assess the clinical potential of α7nAChR in models of psoriasis in comparison to currently available biologics. Surprisingly, nothing is known about the in vivo effect of α7nAChR in models of allergic or toxic contact eczema and atopic eczema. As a future treatment, systemic application of the α7nAChR agents indeed may be superior to topical formulations due to the molecular weight of the α7nAChR agonists such as PHA-543613 or AR-R17779. In addition to these open questions which appear relevant for the design of clinical studies with α7nAChR agonists, there are other areas of terra incognita in α7nAChR biology. They include, for example, the role of α7nAChR in wound healing, acne, melanocyte biology and photocarcinogenesis. Emphasis of our translational research interests on these aspects is likely to generate important new clues to current pathogenetic concepts of several skin diseases and also to promote completely novel treatment and prevention strategies.

ACKNOWLEDGEMENTS
This manuscript was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) to A. S. (STE 2312/1-3).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHORS CONTRIBUTION
MB and AS have designed, written and approved the manuscript.

ORCID
Agatha Stegemann https://orcid.org/0000-0001-5583-1950
Markus Böhm https://orcid.org/0000-0001-7338-7734

REFERENCES
[1] J. H. Steinbach, Ciba. Found. Symp. 1990, 152, 53.
[2] J. Lindstrom, Mol. Neurobiol. 1997, 15, 193.
[3] C. Gotti, F. Clementi, Prog. Neurobiol. 2004, 74, 363.
[4] V. V. Uteshev, Acta Biochim. Biophys. Sin. [Shanghai] 2010, 42, 8.
[5] D. Bertrand, S. Bertrand, M. Ballivet, Neurosci. Lett. 1992, 146, 87.
[6] W. J. de Jonge, E. P. van der Zand, M. F. Bijlsma, D. J. van Westerloo, R. J. Bennink, H. R. Berthoud, S. Uematsu, S. Akira, R. M. van den Wijngaard, G. E. Boeckxstaens, Nat. Immunol. 2005, 6, 844.
[7] J. Arredondo, A. I. Chernyavsky, D. L. Jolkovsky, K. E. Pinkerton, S. A. Grando, FASEB J. 2006, 20, 2093.
[8] H. Wang, H. Liao, M. Ochani, M. Justiniani, X. Lin, L. Yang, Y. Al-Abed, H. Wang, C. Metz, E. J. Miller, K. J. Tracey, L. Ulloa, Nat. Med. 2004, 10, 1216.
[9] R. W. Saeed, S. Varma, T. Peng-Nemeroff, B. Sherry, D. Balakhanesh, J. Huston, K. J. Tracey, Y. Al-Abed, C. N. Metz, J. Exp. Med. 2005, 201, 1113.
[10] R. Hamano, H. K. Takahashi, H. Iwagaki, T. Yoshino, M. Nishibori, N. Tanaka, Shock. 2006, 26, 358.
[11] C. Heeschen, M. Weis, A. Aicher, S. Dimmeler, J. P. Cooke, J. Clin. Invest. 2002, 110, 527.
[12] H. K. Takahashi, H. Iwagaki, R. Hamano, T. Yoshino, N. Tanaka, M. Nishibori, J. Pharmacol. Sci. 2006, 102, 143.
[13] M. Alkondon, E. X. Albuquerque, Prog. Brain. Res. 2004, 145, 109.
[14] R. C. Hogg, M. Raggenbass, D. Bertrand, Rev. Physiol. Biochem. Pharmacol. 2003, 147, 1.
[15] T. Fujii, M. Mashimo, Y. Moriwaki, H. Misawa, S. Ono, K. Horiguchi, K. Kawashima, Front. Immunol. 2017, 8, 1085.
[16] H. Wang, M. Yu, M. Ochani, C. A. Amela, M. Tanovic, S. Susarla, J. H. Li, H. Wang, H. Yang, L. Ulloa, Y. Al-Abed, C. J. Czura, K. J. Tracey, Nature 2003, 421, 384.
[17] M. Bencherif, P. M. Lippiello, R. Lucas, M. B. Marrero, Cell. Mol. Life Sci. 2011, 68, 931.
[18] D. G. Wishka, D. P. Walker, K. M. Yates, S. C. Reitz, S. Jia, J. K. Myers, K. L. Olson, E. J. Jacobsen, M. L. Wolfe, V. E. Groppi, A. J. Hanchar, J. Med. Chem. 2006, 49, 4425.
[19] W. J. de Jonge, L. Ulloa, Br. J. Pharmacol. 2007, 151, 915.
[20] L. Ulloa, Nat. Rev. Drug Discov. 2005, 4, 673.
[21] L. G. Kabelianskaia, Farmakologija I Toksikologija. 1959, 22, 38.
[22] A. M. Weinstein, D. A. Gorelick, Curr. Pharm. Des. 2011, 17, 1351.
[23] D. J. Van Westerloo, I. A. Giebelen, S. Florquin, M. J. Bruno, G. J. Larosa, L. Ulloa, K. J. Tracey, T. van der Poll, Gastroenterology 1822, 2006, 130.
[24] I. Bacher, B. Wu, D. R. Shytle, T. P. George. Expert. Opin. Pharmacother. 2009, 10, 2709.
[25] G. M. Kapalka. Nutritional and Herbal Therapies for Children and Adolescents, 71 Elsevier 2010.
[26] O. Dowling, B. Rochelson, K. Way, Y. Al-Abed, C. N. Metz, Mol. Med. 2007, 13, 576.
[27] J. J. Buccafusco, A. V. Terry, Biochem. Pharmacol. 2009, 78, 852.
[28] J. A. Grizzell, V. Echeverria. Neurochem. Res. 2004, 40, 2032.
[29] W. Kem, F. Sotí, K. Wildeboer, S. LeFrancois, K. MacDougall, D. Q. Wei, K. C. Chou, H. R. Arias, Marine Drugs 2006, 4, 255.
[30] H. Hansen, D. Timmermann, D. Peters, C. Walters, M. Damaj, J. Mikkelson, J. Neurosci. Res. 1810, 2007, 85.
[31] J. Redrobe, Eur. J. Pharmacol. 2009, 602, 58.
[32] E. D. Levin, C. Bettegowda, J. Blasser, J. Gordon, Behav. Pharmacol. 1999, 10, 675.
[33] M. A. Van Maanen, M. C. Lebre, T. van der Poll, G. J. La Rosa, D. Elbaum, J. M. Vervoordeldonk, P. P. Tk, Arthritis Rheum. 2009, 60, 114.
[34] A. Stegemann, D. Flis, W. Ziółkowski, J. H. W. Distler, K. Steinbrink, M. Böhm, J. Invest. Dermatol. 2020, 23, 31399.
[35] A. Kucinski, C. Syposs, S. Wersinger, M. Bencherif, M. K. Stachowiak, E. K. Stachowiak, Schizophr. Res. 2012, 136, 82.
[36] D. J. van Westerloo, I. A. Giebelen, S. Florquin, J. Daalhuisen, M. J. Bruno, A. F. de Vos, K. J. Tracey, J. Infect. Dis. 2005, 191, 2138.
[37] S. Karamihalev, J. Prickaerts, N. P. van Goethem, Behav. Brain Res. 2014, 272, 248.
[38] W. A. Prado, D. K. Segallia, Brain Res. 2004, 1018, 272.
[39] A. Stegemann, A. Sindrilaru, B. Eckes, A. del Rey, S. J. S. Heinick, F. U. Müller, S. A. Grando, B. L. Fiebich, K. Scharrffetter-Kochanek, T. A. Lugner, M. Böhm, Arthritis Rheum. 2013, 65, 792.
[40] A. Stegemann, M. Böhm, Exp. Dermatol. 2016, 25, 994.
[41] L. Vega Lde. E. Muñoz, M. A. Calzado, K. Lieb, E. Candelario-Jallil, H. Gschaidmeir, L. Färber, W. Mueller, T. Stratz, B. L. Fiebich, Biochem. Pharmacol. 2005, 70, 369.
[42] H. Kurzen, H. Berger, C. Jäger, W. Hartschuh, H. Näher, A. Gratchev, S. Goerd, M. Deichmann, J. Invest. Dermatol. 2004, 123, 937.
[43] V. A. Pavlov, M. Ochani, L. H. Yang, M. Gallowitsch-Puerta, K. Ochan, X. Lin, J. Levi, W. R. Parrish, M. Rosas-Ballina, C. J. Czura, G. J. Larosa, E. J. Miller, K. J. Tracey, Y. Al-Abed, Crit. Care. Med. 2007, 35, 1139.

[44] J. E. Macor, D. Gurley, T. Lanthorn, J. Loch, R. A. Mack, G. Mullen, Q. Tran, N. Wright, Med Lett. 2001, 11, 319.

[45] R. L. Papke, H. C. Schiff, B. A. Jack, N. A. Horenstein, Neurosci. Lett. 2005, 378, 140.

[46] T. Stratz, W. Müller, Scand. J. Rheumatol. Suppl. 2004, 119, 59.

[47] W. Müller, T. Stratz, Scand. J. Rheumatol. Suppl. 2004, 119, 44.

[48] J. Arredondo, V. T. Nguyen, A. I. Chernyavsky, D. Bercovich, A. Orr-Urtreger, W. Kummer, K. Lips, D. E. Vetter, S. A. Grando, J. Cell. Biol. 2002, 159, 325.

[49] A. I. Chernyavsky, J. Arredondo, E. Karlsson, I. Wessler, S. A. Grando, J. Biol. Chem. 2005, 280, 39220.

[50] J. Arredondo, L. L. Hall, A. N Doyle, V. T. Nguyen, A. I. Chernyavsky, D. Bercovich, A. Orr-Urtreger, A. L. Beaudet, S. A. Grando, Lab. Invest. 2003, 83, 7.

[51] Z. J. Li, S. B. Park, K. C. Sohn, Y. Lee, Y. J. Seo, C. D. Kim, Y. S. Kim, J. H. Lee, M. Im, J. Dermatol. Sci. 2013, 72, 116.

[52] H. R. Arias, V. E. Richards, D. Ng, M. E. Ghafoori, V. Le, S. A. Mousa, Int. J. Biochem. Cell. Biol. 2009, 41, 1441.

[53] Y. Moriwaki, K. Yoshikawa, H. Fukuda, Y. X. Fujii, H. Misawa, K. Kawashima, Life Sci. 2007, 80, 2365.

[54] F. Chimienti, R. C. Hogg, L. Plantard, C. Lehmann, N. Brakch, J. Fischer, M. Huber, D. Bertrand, D. Hohl, Hum. Mol. Genet. 2003, 12, 3017.

[55] E. N. Lyukmanova, M. A. Shulepko, Z. O. Shenkarev, M. L. Bychkov, A. S. Paramonov, A. O. Chugunov, D. S. Kulkatskii, M. Arvaniti, E. Dolezal, T. Schaer, A. S. Arseniev, R. G. Efremov, M. S. Thomsen, V. Dolezal, D. Bertrand, D. A. Dolgikh, M. P. Kirpichnikov, Sci. Rep. 2016, 6, 30698.

[56] F. A. Kullmann, W. Chet de Groat, D. E. Artim, Botulinum Toxin Therapeutic Clinical Practice and Science 2009, 12, 425.

[57] M. Sticherling, J. Dtsch. Dermatol. Ges. 2019, 17, 716.

[58] D. Singh, A. K. Parihar, S. Patel, S. Srivastava, P. Diwan, M. R. Singh, Pathophysiology. 2019, 26, 103.

[59] Z. Mei, X. Tian, J. Chen, Y. Wang, Y. Yao, X. Li, C. Yang, S. Zhang, C. Xie, Oncol. Rep. 2018, 40, 2287.

[60] K. Kimura, Y. Inaba, H. Watanabe, T. Matsukawa, M. Matsumoto, H. Inoue, J. Diabetes. Investig. 2015, 10, 659.

[61] P. Gisondi, D. Geat, M. Pizzolato, G. Girolomoni, Curr. Opin. Pharmacol. 2019, 46, 90.

[62] K. Furue, T. Ito, G. Tsuji, T. Kadono, M. Furue, G. Ital. Dermatol. Venereol. 2019, 154, 418.

[63] T. S. Wang, T. F. Tsai, Immunotherapy. 2019, 11, 531.

[64] M. Westman, M. Engström, A. I. Catrina, J. Lampa, Scand. J. Immunol. 2009, 70, 136.

[65] O. Abu Zeid, A. Abdel-Aziz, L. A. Rashed, E. R. Said, Clin. Exp. Dermatol. 2019. in press.

[66] H. Tsuji, K. Okamoto, Y. Matsuzaka, H. Iizuka, G. Tamiya, H. Inoko, Genomics 2003, 81, 26.

[67] J. Arredondo, A. I. Chernyavsky, D. L. Jolkovsky, R. J. Webber, S. A. Grando, J. Cell. Physiol. 2006, 208, 238.

[68] S. A. Grando, S. A. Kist, M. Qi, M. V. Dahl, J. Invest. Dermatol. 1993, 101, 2.

[69] A. Stegemann, K. Loser, M. Böhm, Exp Dermatol 2017, 26, 6 (abstract).

[70] J. Wu, E. Guttmann-Yassky, Expert. Opin. Biol. Ther. 2020, 3, 1.

[71] E. Guttmann-Yassky, N. Dhingra, D. Y. Leung, Expert. Opin. Biol. Ther. 2013, 13, 549.

[72] S. Razani-Boroujerdi, R. T. Boyd, M. I. Dávila-García, J. S. Nandi, N. C. Mishra, S. P. Singh, J. C. Pena-Philippides, R. Langley, M. L. Sopori, J. Immunol. 2007, 179, 2889.

[73] A. I. Chernyavsky, J. Arredondo, M. Skok, S. A. Grando, Int. Immunopharmacol. 2010, 10, 308.

[74] B. C. Martel, P. Lovato, W. Bäumber, T. Olivry, Yale J. Biol. Med. 2017, 90, 389.

[75] I. Wessler, T. Reinheimer, H. Kilbinger, F. Bittinger, C. J. Kirkpatrick, J. Saloga, J. Knop, Life Sci. 2003, 72, 2169.

[76] E. M. Peters, A. Michenjo, K. Kupfer, W. Kummer, S. Wiegand, V. Niemeier, N. Potekaev, A. Lvov, U. Gieler, PLoS One 2014, 9, 13552.

[77] B. J. Curtis, J. K. Plichta, H. Blatt, S. Droho, T. M. Griffin, K. A. Radek, Life Sci. 2012, 91, 1070.

[78] K. Scharffetter-Kochanek, P. Brenneisen, J. Wenk, G. Herrmann, W. Ma, L. Kuhr, C. Meewes, M. Waschek, Exp. Gerontol. 2000, 35, 307.

[79] C. C. Zouboulis, E. Makrantonaki, G. Nikolakis, Clin. Dermatol. 2019, 37, 296.

[80] T. A. Lugcr, M. Böhm. J. Invest. Dermatol. 2015, 135, 929.

[81] A. V. Osborne-Hereford, S. W. Rogers, L. C. Gahring, J. Neuroimmunol. 2008, 9, 130.

[82] N. N. Do, S. A. Emir, Curr. Res. Transl. Med. 2016, 64, 185.

[83] J. Marttala, J. P. Andrews, J. Rosenbloom, J. Uitto, Matrix Biol. 2016, 51, 47.

How to cite this article: Stegemann A, Böhm M. Targeting the α7 nicotinic acetylcholine receptor—A novel road towards the future treatment of skin diseases. Exp Dermatol. 2020;29:924–931. https://doi.org/10.1111/exd.14173