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REVIEW ARTICLE

Epithelial and sensory mechanisms of nasal hyperreactivity

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Abstract

"Nasal hyperreactivity" is a key feature in various phenotypes of upper airway diseases, whereby reactions of the nasal epithelium to diverse chemical and physical stimuli are exacerbated. In this review, we illustrate how nasal hyperreactivity can result from at least three types of mechanisms: (1) impaired barrier function, (2) hypersensitivity to external and endogenous stimuli, and (3) potentiation of efferent systems. We describe the known molecular basis of hyperreactivity related to the functional impairment of epithelial cells and somatosensory innervation, and indicate that the thermal, chemical, and mechanical sensors determining hyperreactivity in humans remain to be identified. We delineate research directions that may provide new insights into nasal hyperreactivity associated with rhinitis/rhinosinusitis pathophysiology and therapeutics. The elucidation of the molecular mechanisms underlying nasal hyperreactivity is essential for the treatment of rhinitis according to the precepts of precision medicine.

KEYWORDS
barrier function, mucus secretion, nasal mucosa, sensory nerve, TRP channel

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; AR, allergic rhinitis; IgE, immunoglobulin E; IL, interleukin; K_{ATP}, ATP-gated K^+ current/channel; K_{CA}, Ca^{2+}-gated K^+ current/channel; K_V, voltage-gated K^+ current/channel; NHR, nasal hyperreactivity; NO, nitric oxide; ORAI1, calcium release-activated calcium channel protein 1; STIM1, stromal interaction molecule 1; TREK1, TWIK-related K+ channel 1; TRPV1, transient receptor potential channel vanilloid 1.

1 | INTRODUCTION

Upper respiratory airways conduct air to and from lungs. They also condition and filter the air and play a prominent defensive function against invasive pathogens. The fulfilment of these functions is based on the capability of the upper respiratory tract to behave as a sensory organ, detecting humidity, temperature, airborne particles, and chemicals. Indeed, nasal epithelia hosts chemo-, mechano- and thermo-receptors. Activation of those receptors triggers protective responses, such as sneezing when inhaling pepper, or positive reactions, such as deep breathing when smelling something enjoyable.[1,2] However, in pathological conditions, innocuous stimuli may produce non-adaptative responses, causing inflammation, impairing ventilatory function, and/or generating unpleasant sensations such as pain, itch, and burning. Such phenomenon is referred to as nasal hyperreactivity (NHR, Figure 1), which is defined as "the induction of one or more nasal symptoms such as rhinorrhea, sneezing/itch or obstruction upon encounter of environmental stimuli, like cigarette smoke, temperature/humidity changes, strong odors/fragrances and other irritants."[3] There are other recently identified exacerbating factors, such as emotional stress and physical effort.[4] Frequently, NHR is a manifestation of a pathological condition, affecting 50% of the European population with allergic rhinitis, 60%–70% for non-allergic rhinitis and 30%–40% for chronic rhinosinusitis, being a main symptom in many phenotypes of upper airway pathologies.[5,6]

One of the most important challenges in the clinical management of NHR is the limited availability of standardized diagnostic tools. The diagnose of NHR is nowadays based on questionnaires and not in the direct evaluation of the function of the upper airways.[5] Recently, objective tests such as cold dry air provocations are being recommended for the diagnosis, but without international consensus and with heterogeneity across protocols and pathologies associated with NHR.[7–10] However, some molecular and cellular mechanisms underlying NHR seem to be general, as certain triggering stimuli are not specific of the rhinitis endotype the patient is suffering.[5] This large overlap between the clinical manifestations (phenotypes) of different rhinitis/rhinosinusitis subtypes limits the amount of information that we can deduce about their origin. Consequently, it has been proposed to classify patients based on the pathophysiological mechanisms individually operating in each case (NHR endotypes, also called neurogenic endotypes, which may allow for personalized therapeutic interventions.)[11]

NHR is associated with alterations of the two divisions of nasal innervation: afferent (somatosensory systems) and efferent (sympathetic or parasympathetic motor systems).[4,12] Nevertheless, it seems appropriate to consider that hyperreactivity is the pathophysiological result of multiple processes not limited to aberrant neuronal function. In other words, some NHR symptoms might not be directly caused by neuronal dysfunction. For example, epithelial function alterations or the potentiation of effector cells (mucus secretory cells, vasculature, or immune cells) may produce NHR. Table 1 summarizes the cellular pathways implicated in NHR pathophysiology.

In the following, we will discuss how different molecular and cellular pathways are implicated in the pathophysiology of NHR. The purpose of this review is to recapitulate the cellular and molecular mechanisms determining NHR, limiting the scope to the implications of epithelial cells and afferent neurons. For an overview of the roles of other cell types in NHR and rhinitis, we refer the reader to the literature on immune cells,[13,14] efferent innervation, and vasculature.[11,15] The analysis of the literature led us to propose that for a better understanding of rhinitis pathophysiology and endotypes, the term NHR should be split in at least three categories: (1) deficient barrier function, (2) hypersensitivity, referring to enhanced sensory function, and (3) hyperresponsiveness, indicating for potentiated response mechanisms.

2 | EPITHELIAL AND SOMATOSENSORY PATHOPHYSIOLOGY IN NHR

2.1 | Epithelial cells

The nasal epithelium is formed by different cells (ciliated, goblet, and basal cells) and it is the first tissue exposed to environmental stimuli causing NHR.[16] It has different protective functions (Figure 2): (1) acts as a physical barrier limiting the penetration of pathogens, particles, and chemicals; (2) transports water; (3) produces mucus; (4) supports mucociliary clearance, and (5) releases mediators that regulate the function of other structural and immune cells.

2.1.1 | Barrier function

The barrier function is supported by different intercellular protein complexes, such as tight junctions, adherent junctions, and desmosomes.[16,17] Tight junctions control ions, water, and macromolecules transport through the epithelium and are located in the apical zone of epithelial cells. Diverse environmental factors disrupt the function of tight junctions, such as proteases present in dust mite feces and in the pollen of some plants, cigarette smoke and microparticles generated by Diesel combustion.[18–22] Also, several endogenous factors can affect tight junctions, such as cytokines IL-4 and IL-13 derived from type Th-2 immune activation.[23]

Barrier function is decreased in patients with allergic rhinitis induced by dust mites.[24] Interestingly, patients with non-allergic rhinitis do not exhibit defects in barrier function of the nasal epithelium.[25] Moreover, histamine and nasal secretion of allergic patients induce a fast deterioration of the barrier function of cultured nasal epithelial cells, which is not the case for secretions of non-allergic rhinitis patients.[25] This indicates that inflammatory mediators present in allergic rhinitis modulate barrier function, and that in non-allergic rhinitis, the associated molecular signaling has no clear relationship with it.[25] From a mechanistic point of view, these results indicate that NHR does not necessarily require a deficit in barrier function to be present, as non-allergic rhinitis patients can also

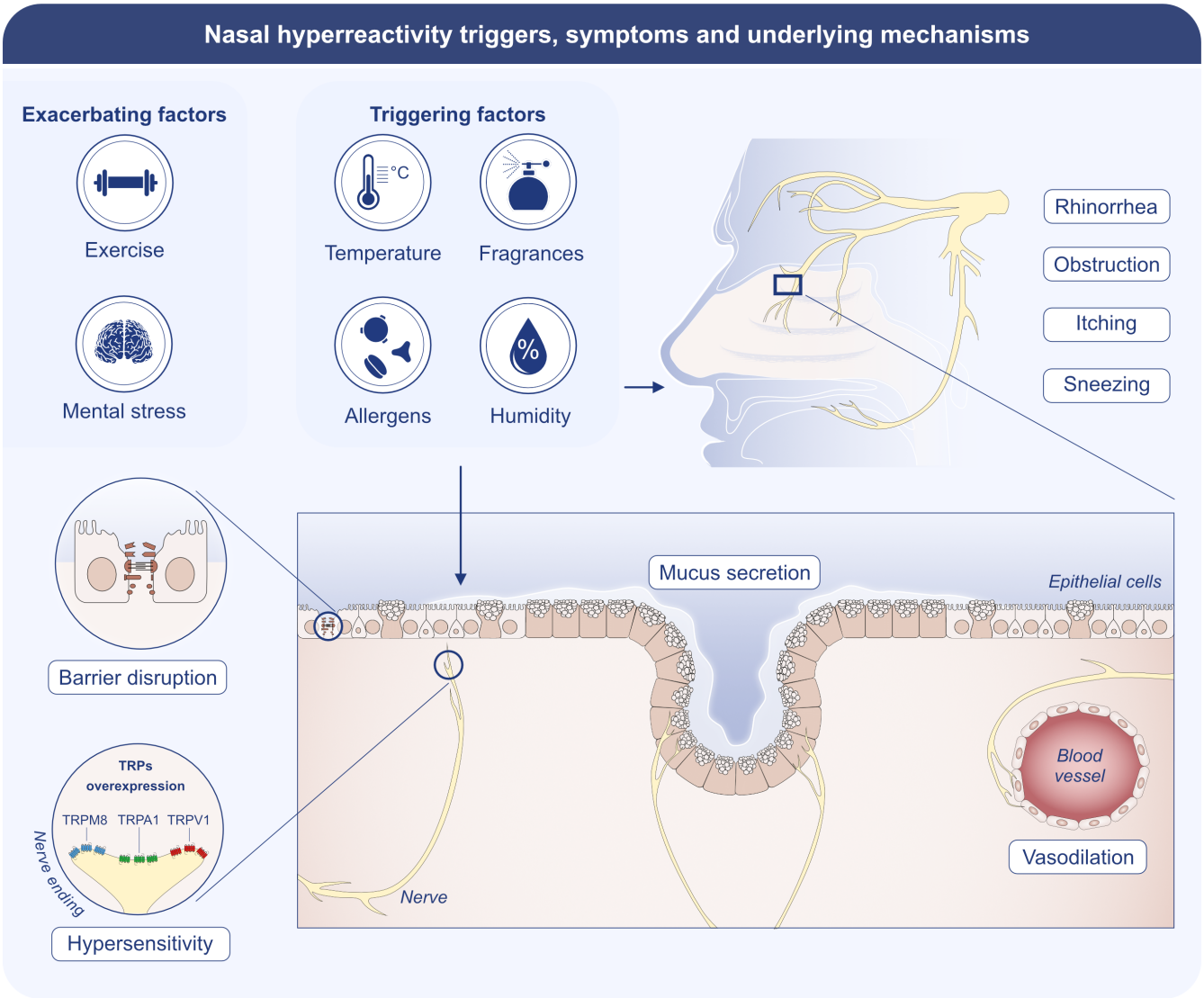

FIGURE 1 Nasal hyperreactivity (NHR) triggers, symptoms and underlying mechanisms. Environmental stimuli such as fragrances, allergens and changes in temperature and humidity can trigger NHR. These factors cross the epithelial barrier and are detected by the trigeminal nerve terminals. Nerve terminals transduce stimuli through multiple molecular mechanisms, such as the activation of transient receptor potential (TRP) cation channels, which leads to nasal sensations such as itch. In addition, this triggers antidromic afferent nerve and efferent reflex mechanisms leading to mucus secretion and vasodilation, ultimately producing rhinorrhea, sneezing and nasal obstruction. Either barrier dysfunction, hypersensitivity or hyperresponsiveness may result in NHR manifestation, making it a common symptom in distinct pathologies. Factors such as exercise and mental stress can lead to NHR via yet unclear mechanisms

display NHR symptoms. This highlights an important consideration: because many pathophysiological pathways can lead to NHR, generalization of mechanisms found in a specific condition (i.e., allergic rhinitis) to others could be misleading.

The TWIK-related K^+ channel 1 (TREK1), expressed in nasal epithelium, has been directly related with NHR in rhinitis.[16] In general, TREK1 regulates K^+ concentration and resting membrane potential.[26] Nasal epithelial cells of rats and patients suffering from allergic rhinitis display lower levels of TREK1 mRNA and protein expression. Moreover, the inflammatory cytokine IL-4 suppresses TREK1 expression through activity upregulation of the histone deacetylase 1 (HDAC1). IL-4 and IL-13 interference over the histone deacetylases' activity could result in an arrest of the epithelial cells in a pathological state, chronifying the disease condition.[16] Indeed, IL-4 impairment of the barrier function could be prevented by blocking HDAC1 in rats, which suggests a role for TREK1 in maintaining barrier function in allergic rhinitis.[27] Furthermore, antigen-specific immunotherapy restored the diminished levels of TREK1 in allergic rhinitis patients, an effect that could be potentiated with a combined treatment with the probiotic *Clostridium butyricum*.[28]

2.1.2 | Water transport

Epithelial apical surface hydration is fundamental for the correct functioning of the airways.[29] In the nasal epithelium, water transport relays

TABLE 1 Cellular pathways implicated in the pathophysiology of NHR

Cell	Alteration group	Function	References
Ciliated epithelial	Barrier function Hypersensitivity	− barrier function	23,27,28
		− ciliary beat	36,40,42-45
		? water transport	30–32
		+ cell-to-cell signaling	45,46,48,76
Sensory neurons	Hypersensitivity	+ excitability	15,85,93,94
		+ stimulus detection	59,65
		+ cell-to-cell signaling	82
Efferent neurons	Hyperresponsiveness	+ excitability	15
		+ effector activation	35,37
Immune	Hyper-sensitivity/ responsiveness	+ stimulus detection	23,81,99
		+ barrier function	25
		+ cell-to-cell signaling	33,35,37,99,104
Goblet	Hyperresponsiveness	+ mucus secretion	35,37,76
Submucosal glands	Hyperresponsiveness	+ mucus secretion	35,37,103
Vascular	Hyperresponsiveness	+ vasodilation	82
Basal?	Hypersensitivity/ responsiveness	+ cell-to-cell signaling	50
Club cells?	Hypersensitivity/ responsiveness	+ cell-to-cell signaling	51
Neuroendocrine?	Hypersensitivity/ responsiveness	+ cell-to-cell signaling	16
Solitary chemosensory?	Hypersensitivity/ responsiveness	+ cell-to-cell signaling	16

Note: The signs "+" and "−" indicate increased and decreased function, respectively. "?" indicates potential candidates to be implied in NHR or rhinitis, as there are conflicting results regarding the direction of the change.

mainly in the activity of aquaporin water channels, particularly AQP5, located at the apical membrane of epithelial cells.[30] AQP5 and AQP4 are overexpressed in the cytoplasm of epithelial glandular cells of patients suffering from chronic rhinosinusitis with and without polyps, respectively.[31] However, another study contradicts this affirmation, finding reduced AQP5 expression in patients with nasal polyps when compared to those without polyps or control subjects. This study demonstrates that while AQP5 expression in polyp epithelial cells is reduced, the expression in the sub-epithelium is increased.[32] The authors of the work that found increased AQP5 expression argue that this discrepancy could be due to the low number of subjects included in the other study or because they analyzed specific localization of AQP5 expression, while the other study analyzed labelling in the entire surface of the epithelium. Noteworthy, histamine reduces AQP5 expression in cultured human nasal epithelial cells, being this effect reversed by the antihistaminic chlorpheniramine.[33]

In addition to contributing to humidification of inhaled air, water is a critical regulator of ciliary beat.[34] Moreover, it may contribute to a semi-aqueous barrier, limiting the diffusion of volatile hydrophobic organic compounds, such as essential oils present in perfumes and cosmetic products. Therefore, an airway with a deficient humidification of the epithelium may be hyperreactive to these compounds.

2.1.3 | Mucus secretion

Mucus is secreted by goblet cells and subepithelial glands and is fundamental for airway defense. First, mucins, which are high molecular weight glycoproteins that give viscoelastic properties to mucus, facilitate ciliary beat function. Additionally, mucus contains antioxidants, antiproteases, and antimicrobial factors, which protect against pathogens and pollutants.[35,36]

Mucins' expression and secretion, specifically MUC5AC, is increased by proinflammatory agents, both exogenous (pollutants, microbial products) and endogenous (cytokines, retinoic acid, epidermal growth factor, ATP, etc.). In the context of NHR, exacerbated mucin secretion determines the symptom rhinorrhea, present in all rhinitis and rhinosinusitis subtypes. It limits ciliary beat that together with tissue inflammation reduces mucociliary clearance, facilitating the accumulation of proinflammatory factors. This produces a positive feedback loop sustaining mucus hypersecretion, particularly in chronic rhinosinusitis.[35,37] In allergic rhinitis, histamine upregulates mucin secretion through stimulation of cholinergic nervous terminals and cysteinyl leukotrienes.[35,37]

Finally, the expression of the transient receptor potential vanilloid 4 channel (TRPV4) is increased in sinonasal mucosal biopsy samples of patients suffering from chronic rhinosinusitis without polyps.[38]

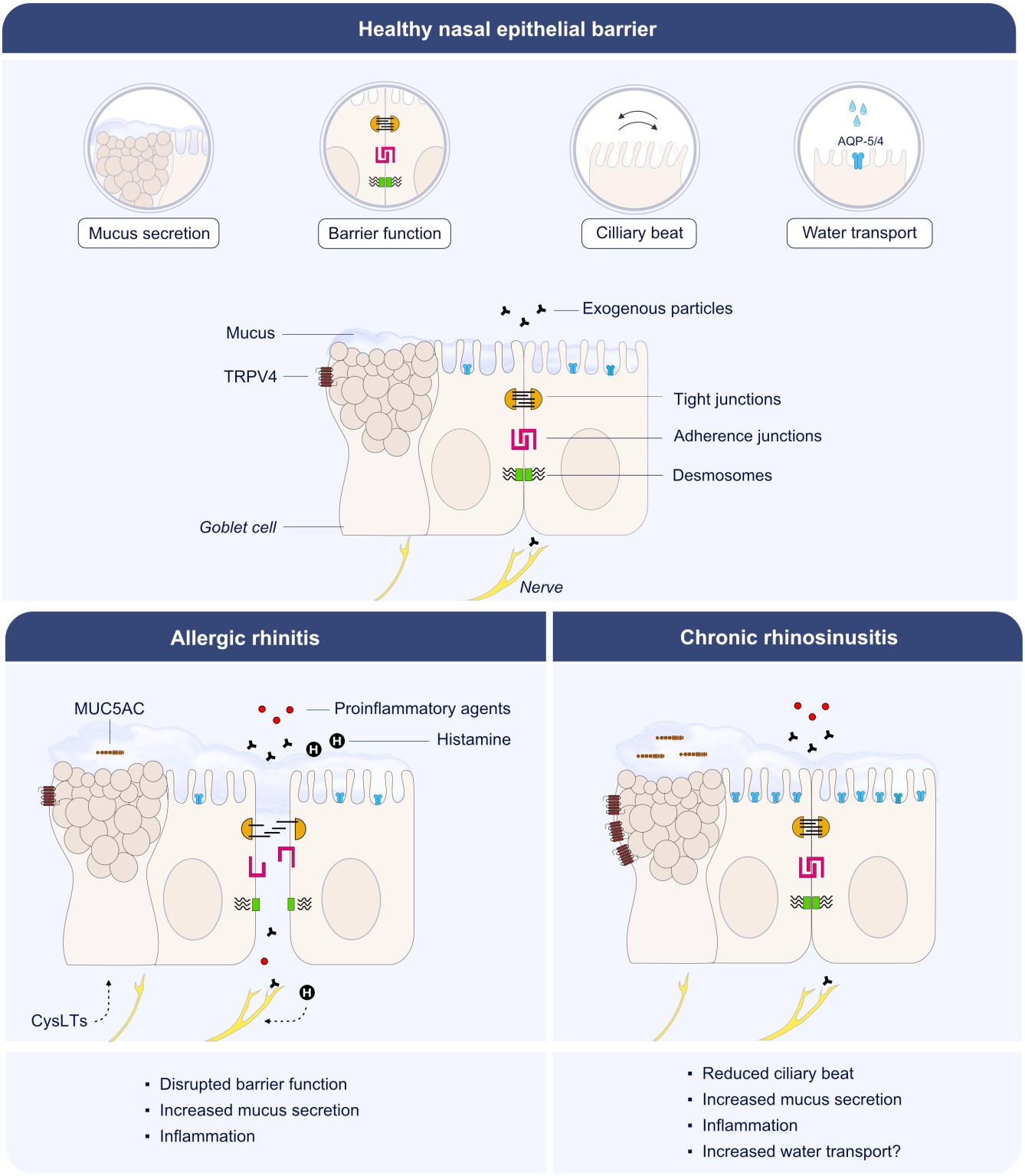

FIGURE 2 Alterations of epithelial functions in allergic rhinitis and chronic rhinosinusitis leading to NHR. States of the nasal epithelium in normal condition, allergic rhinitis and chronic rhinosinusitis. The nasal epithelium produces mucus, acts as a barrier against external stimuli, produces and supports ciliary beat and mediates water transport. In allergic rhinitis, inter-cellular junctions supporting the barrier function become dysfunctional under the action of inflammatory mediators such as histamine, allowing external molecules to reach sensory nerve terminals, favoring NHR. The expression of mucins (MUC5AC) and mucus secretion produced by goblet cells are increased in all forms of rhinitis and rhinosinusitis, leading to rhinorrhea. Ciliary beat is thought to be diminished in chronic rhinosinusitis due to mucus stagnation, therefore disrupting mucociliary clearance. The TRPV4 cation channel expression is increased in patients with chronic rhinosinusitis without polyps, and could mediate mucus production via its function as viscosity sensor. There are controversial results regarding the expression of aquaporins (AQP) in chronic rhinosinusitis, although they seem to be upregulated in this condition

In hamster oviductal ciliated cells, TRPV4 has been associated with the detection of mucus viscosity, modulating ciliary beat.[39] In human sinonasal mucosa, TRPV4 is not expressed in ciliary cells, excluding that role.[38] However, this viscosity detector channel could instead be expressed in mucus secretion cells, regulating mucus production.

2.1.4 | Ciliary beat

Ciliary beat is crucial for nasal mucosa protection. Nevertheless, no studies relate ciliary beat function with NHR in rhinitis/rhinosinusitis. It is known that patients with primary cilia dyskinesia suffer from chronic rhinosinusitis[36,40] and, consequently, may manifest NHR. Their responses to adrenergic, cholinergic, purinergic, and mechanical stimuli are altered, and ciliary beat frequency could be altered as well.[41] While it is not known to what extent mucus stagnation contributes to NHR symptoms, the accumulation of tissue debris, pathogens, pollutants and proinflammatory mediators may contribute to its exacerbation. Moreover, some factors and stimuli associated to NHR such as aging, low temperatures, hyperventilation associated with physical effort and environmental pollutants have negative effects on ciliary movement.[42–44]

2.1.5 | Other secretory functions

Epithelial cells release mediators that regulate their own function and the activity of other cells. Ciliated cells release ATP to the lumen through pannexin 1 channels (PANX1) and Ca^{2+} homeostasis modulator 1 (CALHM1) when they are mechanically stimulated by breathing, sneezing, or coughing, by osmotic shock or by shear stress. Thereafter, extracellular ATP stimulates P2X and P2Y purinergic receptors, increasing intracellular Ca^{2+} concentration and thereby the ciliary beat frequency.[45] Extracellular ATP is also a damage-associated signal that stimulates goblet cells, nociceptors, and immune cells. However, the role of extracellular ATP in rhinitis or NHR has not been explored.

Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are also released by epithelial cells. In response to viral infections, nasal epithelial cells from mice and patients produce H_2O_2 through the enzyme dual oxidase (Duox2).[46] This innate immune response limits viral infection, but induces oxidative stress in the tissue, which may activate the transient receptor potential ankyrin 1 cation channel (TRPA1) expressed in nociceptors, producing pain and neurogenic inflammation.[47] NO is produced by nasal epithelial cells stimulated by bacterial lipopolysaccharides (LPS) through activation of TRPV4.[48] NO has antimicrobial and antiviral direct effects and modulates several functions of the airways, including ciliary beat, mucus secretion and vasodilation. On the other hand, it contributes to tissue damage during inflammation.[49] As discussed below, H_2O_2 and NO may increase responses to cold through TRPA1 and TRPM8 sensitization.

Basal epithelial cells secrete other immune mediators such as RNAases, defensins, cytokines, and chemokines,[50] and club cells produce secretoglobin SCGB1A1.[51] Although it is known that both cell types play a role in tissue regeneration, their functions in NHR remain unexplored.

Neuroendocrine cells have not been described in nasal mucosa, but in lower respiratory airways, they function as chemoreceptors, interacting with goblet cells, immune cells and with the sympathetic and parasympathetic nerves.[16] Also, solitary chemosensory cells detect chemical compounds through mechanism based on bitter taste receptors and, when stimulated, release defensins (antimicrobial peptides) and IL-25, associated to Th-2 immune responses.[16]

2.2 | Somatosensory innervation

The whole subepithelial space, including glands and blood vessels, is densely innervated by afferent somatosensory nerve endings, which functions are detecting thermal, mechanical, and chemical stimuli, and releasing mediators that interact with other cells.[15] This nerve endings are the peripheral processes of primary sensory neurons located at the trigeminal ganglion and can be divided into innocuous and noxious stimuli detectors (nociceptors). In the upper airway, nociceptors are mostly C-type fibers: they are unmyelinated, have small caliber and low conduction velocity. This kind of fibers is typically sensitive to many chemical and physical stimulation modalities (polymodality).[15]

Afferent terminals transduce the energy of the stimuli into electrical signals that can be conducted and interpreted by the central nervous system, resulting in adaptative behaviors (defensive or aversive, such as coughing) and local tissue responses (neurogenic inflammation). Sensory nerves express molecular sensors (such as TRP channels) that are activated by external and endogenous stimuli. This leads to cation entry through the plasma membrane that results in a depolarization of the membrane potential of the sensory nerve. This depolarization is amplified by the opening of voltage-gated Na^+ channels (Na_V), producing action potential firing, whose frequency is the variable encoding the stimulus properties. Conversely, K^+ channels opening hyperpolarizes the membrane, opposing to excitability in resting conditions (i.e., in the absence of stimuli), and producing repolarization of the action potential, permitting the recovery of Na_V channels from inactivation. This simple scenario is complicated by the diverse underlying molecular determinants. For example, sensory neurons express different Na^+, K^+, Ca^{2+}, ASIC, P2X, and TRP channels, in addition to mechano-sensitive channels (i.e., PIEZOs). Noteworthy, alterations in the mechanisms regulating the expression and function of these channels may result in the development of pathological nerve activity (Figure 3). Therefore, they are considered potential therapeutic targets.

2.2.1 | TRP channels

Since their original cloning from the fruit fly (*Drosophila melanogaster*), TRP channels have received great attention due to their ubiquitous expression, their capability to permeate Ca^{2+} and other

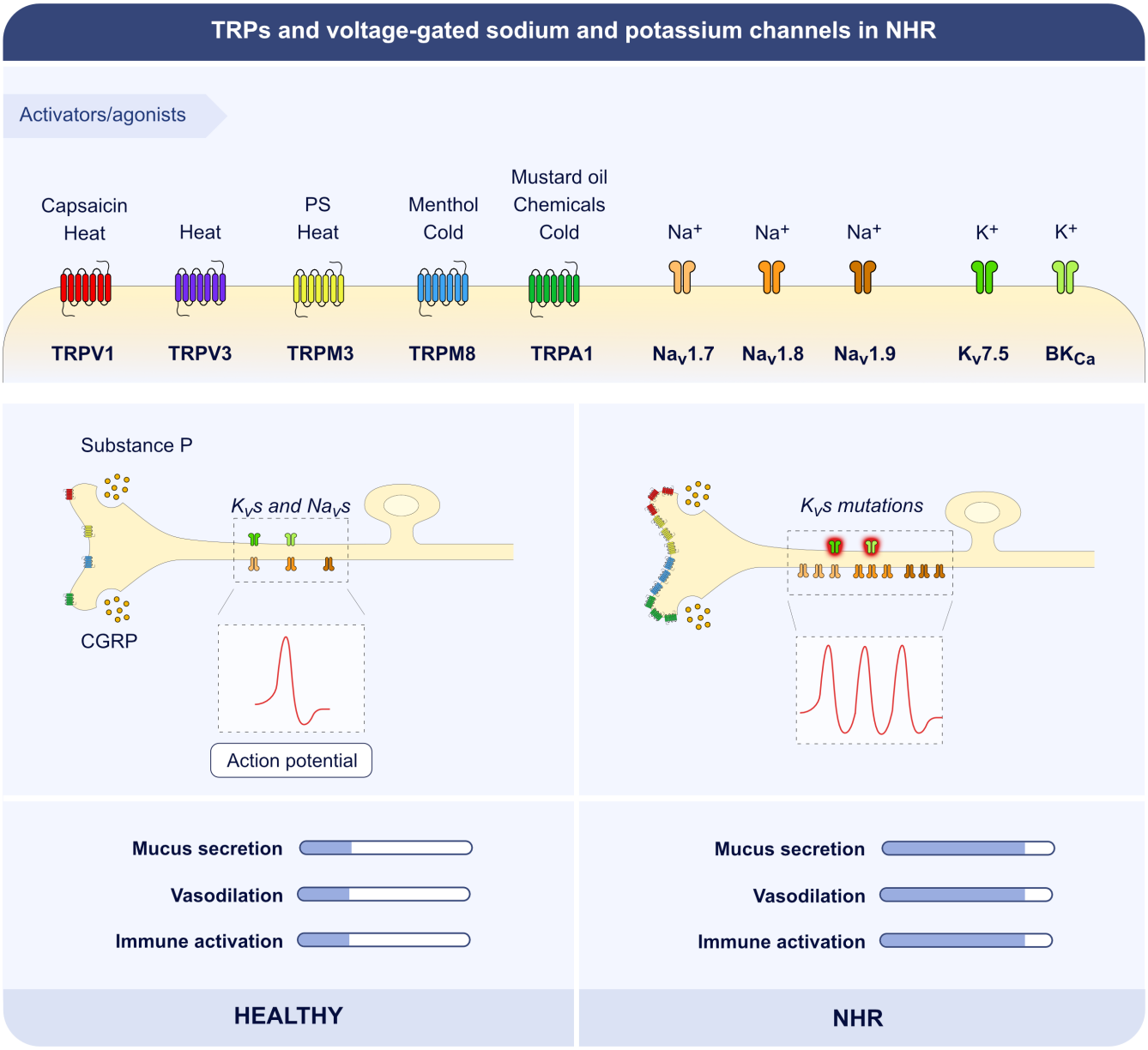

FIGURE 3 Roles of transient receptor potential (TRP) channels and voltage-gated Na^+ channels (Na_V) and K^+ (K_V) channels in nasal mucosa physiology and NHR pathophysiology. TRP channels transduce stimuli into intracellular signals, such as membrane depolarization and increase in intracellular Ca^{2+} concentration. TRPV1, TRPA1, TRPM8 and TRPM3 are expressed in sensory nerve terminals, not necessarily in the same neuronal populations, whereas TRPV3 is expressed in epithelial cells and eosinophils. TRP-mediated signaling in afferent nerve terminals regulates vasodilation, immune activation and mucus secretion via antidromic afferent and efferent reflexes. The depolarization induced by TRP channel activation is transduced into action potentials via the subsequent activation of Na_V and K_V channels expressed in the sensory nerve terminals. The pattern of action potential firing encodes the sensory information that is conveyed to the Central Nervous System, where conscious perceptions such as the sensations of pain and itch are ultimately generated. In NHR, TRP and Na_V channels are upregulated and some K_V isoforms are mutated. This results in increased immune activation, vasodilation, and mucus secretion. These changes also enhance primary sensory neurons excitability, increasing action potential firing and generating the sensory symptoms characteristic of NHR. Not all the depicted changes are documented in all pathologies featuring NHR (see main text for detailed description). PS: pregnenolone sulfate

cations, and their consequent ability to regulate Ca^{2+} signaling and membrane potential. They show high conservation between species and are conformed by homo- and hetero- tetrameric protein assemblies with six transmembrane segments forming a pore. Attending to their amino acidic sequence homology, they are classified into six families in mammals: TRPC (*Canonical 1-7*), TRPV (*Vanilloid 1-6*), TRPM (*Melastatin 1-8*), TRPA (*Ankyrin 1*), TRPP (*Polycystin 1-3*), and TRPML (*Mucolipin 1-3*).[52,53]

Among the TRP channels expressed in the afferent nerves, TRPV1 and TRPA1 have received greater attention in the context

of rhinitis (Figure 3). They are part of cell signaling cascades mediated by G-protein-coupled receptors and are directly activated by thermal, mechanical, and chemical stimuli, including a wide variety of noxious exogenous compounds and molecules associated with tissue damage.[15,47,54] Importantly, the activation of these channels depolarizes the nerve and induces release of neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P, causing vasodilation, increased capillary permeability, plasma extravasation and regulation of mast cell function.[55]

Capsaicin, a specific TRPV1 agonist, has been used as a therapeutic agent in patients with non-allergic, non-infectious rhinitis for more than 30 years.[4,56] Capsaicin instillation induces defunctionalization of nerves expressing TRPV1, reducing NHR and associated symptoms in humans.[57] Patients with idiopathic rhinitis (a subgroup of non-allergic rhinitis[58]) show increased *TRPV1* RNA expression in the nasal mucosa and high levels of substance P.[59] Capsaicin treatment reduces symptoms and hyperreactivity produced by cold dry air in humans.[59] Subsequent studies demonstrated that idiopathic rhinitis patients display increased sensitivity to intranasal administration of allyl isothiocyanate (mustard oil), which is a classical chemical irritant that activates TRPA1,[60] as well as TRPV1[61–63] and TRPM8,[64] and produces neurogenic inflammation. This hyperreactivity could be reverted applying intranasal capsaicin.[65] More recently, it was demonstrated that daily low dose of capsaicin could be as effective for patients as the classic high-dose acute treatment, that must be performed in controlled clinical settings.[66]

Regarding allergic rhinitis, the involvement of TRPV1 and the effectivity of capsaicin as a treatment are dubious. Capsaicin stimulation induces more symptoms and pain during allergy season, suggesting a sensitization to this compound during allergic inflammation in those patients.[67] In patients with dust mite allergic rhinitis, symptoms are not reduced by intranasal capsaicin, although responsiveness to histamine is.[68] Moreover, TRPV1 inhibition does not reduce spontaneous nasal symptoms triggered by allergens in allergic rhinitis patients.[69,70] Interestingly, the combination of the antihistaminic azelastine (which reduces TRPV1 expression together with its H1 antagonist effect) and the corticosteroid fluticasone manages to reduce nasal symptoms, NHR, and substance P levels in secretions of dust mite-allergic rhinitis patients.[71] In conclusion, TRPV1 inhibition may be useful to reduce neurogenic inflammation, but it's efficacy in allergic conditions remains unclear. This could be due to the higher complexity of the pathophysiology of allergic rhinitis compared to that of idiopathic rhinitis, being the former related to Th-2 inflammatory mechanisms and not only to neurogenic inflammation.

TRPA1 is another channel with great potential implication in NHR pathophysiology. TRPA1 is expressed both in afferent nerves and epithelial cells of the airways, and is activated by a wide variety of external chemical compounds (electrophilic, alkaloids, cannabinoids, alcohols, menthol and other terpenes, H_2O_2, NO, ozone, LPS, etc...) and by endogenous agents produced during tissue damage, as well as by intense cooling.[47] Patients with idiopathic rhinitis display increased *TRPA1* RNA levels in the nasal mucosa and have low irritation threshold for the TRPA1 agonist allyl isothiocyanate.[65] Furthermore, TRPA1 is one of the candidates mediating cold responses in the airway, and, in consequence, it may be the molecule responsible for naso-constrictor response to cold dry air that is used as a diagnostic tool for NHR in rhinitis.[72] Moreover, TRPA1 activity can be potentiated by mediators produced in an inflammatory context.[47,54] Pharmacological blockade of TRPA1 channels present in trigeminal sensory neurons reduces the inflammatory response to the allergic challenge, as well as the activity of nociceptors in the inflamed area in mice.[73] A relevant example for NHR in rhinitis is that the cold-induced activation of human TRPA1 is potentiated by the oxidant agent H_2O_2[74] and it is expected that the same happens with NO.

TRPM8 is another possible mediator of hyperreactivity to cold (Figure 3). Morphological studies have revealed TRPM8 immunoreactivity in subepithelial nerves and surrounding human nasal mucosa vasculature.[75] Activation of TRPM8 by cold or menthol is proposed as the mechanisms triggering mucus production,[76] and may explain rhinorrhea and postnasal drip. On the other hand, no differences in TRPM8 expression were found between rhinitis patients and healthy controls.[75,76] Noteworthy, TRPM8 is also associated with empty nose syndrome,[77] as is proposed to act as an air flux detector through evaporation-induced cooling. This evaporation-sensing role has been demonstrated for the ocular surface,[78] which is also a trigeminally-innervated mucosa. Furthermore, in the ocular surface, TRPM8 acts as an osmosensor and regulates tearing and blinking.[79] H_2O_2 induces TRPM8 upregulation and potentiation in mouse urothelium,[80] and this may occur as well in the nasal epithelia during rhinitis.

TRPV3 expression is increased in eosinophils and in the epithelium of polyps of patients suffering from eosinophilic chronic rhinosinusitis (Figure 3).[81] Furthermore, TRPV3 expression was correlated with refractoriness of rhinitis symptoms after surgery.[81] Interestingly, TRPV3 is not expressed in blood circulating eosinophils, being transcribed *de novo* in the context of eosinophilic chronic rhinosinusitis, suggesting a pivotal pathophysiological relevance for this molecule.[81]

Lastly, recent studies in mice demonstrate that TRPM3 activation through the neurosteroid pregnenolone sulfate produces vasodilation of resistance arteries, mediated by CGRP release by the perivascular afferent nerve terminals.[82] This suggests that TRPM3 might be implicated in rhinitis through modulation by endogenous mediators (Figure 3).

2.2.2 | Voltage-gated Na^+ channels

Voltage-gated Na^+ channels (Na_V or VGSC) are composed by an α subunit and associated β subunits. The α subunit has six transmembrane segment and form a pore with Na^+ selectivity, that contains the voltage sensors. Peripheral sensory neurons (afferent innervation) are one of the cellular subtypes expressing the richest variety of Na_Vs: that is, $Na_V1.1$, $Na_V1.6$–1.9. Moreover, in pathological conditions, the embryologically expressed Na_V 1.3 is

re-expressed.[83] Na_Vs trigger action potential generation in neurons when a depolarizing threshold is reached, usually upon the depolarization induced by transducer channels such as TRPs. Therefore, Na_Vs play a crucial role in normal sensation and pathologies featuring aberrant perceptions such as chronic pain and itch (reviewed in [ref. 84]).

Na_V channels are candidates to be common pathophysiological players sustaining NHR across distinct rhinitis endotypes (Figure 3). Na_V1.7–1.9 are characteristic of sensory neurons. Their expression is increased in biopsy samples from both allergic and non-allergic rhinitis patients, independently of the level of inflammation presented.[85] Furthermore, their expression is upregulated by many mediators involved in rhinitis, such as nerve growth factor (NGF).[85] Moreover, Na_V1.9 is directly activated by NO in mice, a key rhinitis mediator.[86] Finally, neuropathic and inflammatory conditions alter the expression of these channels, triggering ectopic firing of the fibers through destabilization of the membrane voltage leading to hyperexcitability.[87] This instabilities of the membrane voltage are sustained mainly by tetrodotoxin-resistant channels Na_V1.8 and 1.9 in mouse trigeminal neurons[88] (reviewed in ref. [89]). As no functional or clinical studies of these channels has been performed in the rhinitis field, they represent an open niche for future research.

2.2.3 | *K^+ channels*

K^+ channels are one of the most diverse molecular ion channel superfamilies known. Briefly, K^+ channels regulate the resting membrane potential and action potential firing in neurons[90] and influence both hydration and mucus clearance in epithelial cells.[91,92] They are a promising therapeutic target for NHR treatment (Figure 3). In a pediatric cohort, mutations in two K^+ channel encoding genes, whose protein product are present in the airways, were correlated with the development of chronic rhinosinusitis: *KCNMA1* (encoding for BKca, a Ca^{2+}-activated K^+ channel) and *KCNQ5* (encoding for the K_V7.5 voltage-gated potassium channel).[93] Those results have multiple implications, being the most outstanding that genetic traits associated with rhinitis vary between ethnicities and that K^+ could be mechanistically related to the NHR in chronic rhinosinusitis. Coherently, tetraethylammonium (TEA, a blocker of Ca^{2+}-activated and voltage-activated K^+ channels), as well as glibenclamide (an ATP-sensitive K^+ channel inhibitor), reduce the potentiated vasodilation induced by NO in an allergic rhinitis rat model.[94] This suggests that such channels are implicated in vascular-related NHR symptoms, and maybe in the effects produced by ATP release. Indeed, the inhibition of the Ca^{2+}-activated K^+ channel K_{Ca}3.1 by the specific antagonist TRAM-34 applied intranasally reduced sneezing, nasal rubbing, epithelial cell proliferation, eosinophil infiltration and decreased cytokine expression in the nasal lavage fluid in a mice model of allergic rhinitis.[95] Interestingly, the use of this inhibitor also reduces inflammation induced by allergic asthma in mouse and human cells.[96] Altogether, K^+ channels appear to be important players in rhinitis pathophysiology and are therefore promising therapeutic targets for NHR.

2.2.4 | Ca^{2+} release-activated Ca^{2+} channels: ORAI1

Ca^{2+} is a key factor in cellular excitability and one of the most important intracellular second messengers. The research on the implications of Ca^{2+} channels in the rhinitis context has been centered mainly around Ca^{2+} release-activated Ca^{2+} channel protein 1 (ORAI1), a selective Ca^{2+} channel that is activated upon depletion of intracellular Ca^{2+} stores. ORAI1 has a crucial role in short- and long-term response of immune system cells, is expressed in nasal mucosa and is upregulated in murine models of allergic rhinitis.[97] In these models, inhibition of ORAI1 expression decreases NHR and reduces inflammatory mediators in the nasal lavage serum in allergic mice.[98] Some specific signaling has been reported, such as ORAI1 being increased in nuocytes originated from allergic nasal-associated lymphoid tissue. Indeed, ORAI1 expression in nuocytes has been implicated in the exacerbated response to IL-33 in those mice.[99] Following this line, there is preliminary evidence that compounds disrupting ORAI1 signalization alleviate NHR symptoms in murine allergic rhinitis models, such as aminoethoxydiphenyl borate (2-APB), natural compounds extracted from *Flos magnoliae* (through fargesin and other active principles), anti-ORAI1 antibodies and short hairpin RNA, opening a promising research direction to develop new treatments.[98,100–102]

2.2.5 | Implication of other channels in rhinitis/rhinosinusitis

The list of channels expressed in afferent nasal nerves that is potentially implicated in upper airway pathophysiology is long. In it, there are not only TRPs, but also mechano-sensitive channels and channels regulating excitability of the nerve terminals. It draws our attention that the molecular determinants of mechanical hypersensitivity in rhinitis are largely unknown and there are only a few studies implicating mechanosensitive nerve fibers in this pathology.[27,28] Even more outstanding is that the possible implication of voltage-gated Na^+ channels and K^+ channels has been barely explored.[15] Nevertheless, preliminary evidence is available for some other channels. For example, CLC-3, a Cl^- channel, is expressed in epithelium and submucosal glands and is upregulated in nasal mucosa from patients with allergic rhinitis.[103] Also, acid sensing ion channels (ASICs) have been related to allergic rhinitis NHR symptoms, as low pH produces a rise in nasal fluid secretion that is disrupted by an ASICs inhibitor. Furthermore, ASIC-3 expression is upregulated in the nasal epithelium of these patients, which seems to be induced by eosinophil peroxidase signaling.[104]

3 | NHR, RELATED DISEASES AND TREATMENT

NHR is increasingly recognized as a presenting symptom of inflammation in both the nasal and the sinonasal mucosa. Indeed, up to 2/3 of rhinitis patients, as well as rhinosinusitis patients may

TABLE 2 List of NHR treatments, model in which they have been studied, proposed mechanistical rationale for their effects, measured outcomes and references

Treatment	Studied model	Rationale	Outcomes	References
Antigen-specific immunotherapy combined with probiotic *C. butyricum*	AR patients	Butyrate produced by the probiotic inhibits HDAC1, which inhibits TREK1 expression	- Restored TREK1 levels - Reduced NHR symptoms - Reduced Th2 circulating markers	28
Intranasal capsaicin: High, single dose or daily low dose	Non-allergic rhinitis patients	Defunctionalization of nerve ending expressing TRPV1, mainly nociceptors. TRPV1 is upregulated in these patients	- Reduced NHR symptoms - Restored sensitivity to physical and chemical stimulation - Reduced TRPV1 expression	4,56,57,59,65,66
Intranasal capsaicin	AR patients	Defunctionalization of nerve ending expressing TRPV1, mainly nociceptors	- Reduced histamine-evoked response - No effect over spontaneous symptoms	68–70
Selective TRPV1 inhibitors	AR patients	Direct blockade of TRPV1-mediated transduction, without affecting nerve ending integrity	- No effect over allergen-induced symptoms	69,70
Intranasal azelastine + fluticasone (antihistaminic and corticosteroid)	AR patients	- Histamine receptor 1 antagonism - Anti-inflammatory - Azelastine reduces TRPV1 expression	- Reduced NHR symptoms - Reduced substance P expression	71
Tetraethylammonium (K_V and K_{Ca} inhibitor) Glibenclamide (K_{ATP} inhibitor)	AR rats	NO induces increased vasodilation and activates K^+ channels in AR patients. Blocking them could attenuate the vasodilation that is thought to cause nasal obstruction	- Reduced NO-induced vasodilation	94
TRAM-34 (K_{CA}3.1 inhibitor)	AR mice	Influx of Ca^{2+} is pivotal in the activation of eosinophils and dendritic cells. For it to be possible, electrical driving force is maintained by K_{CA}3.1. Blocking it could prevent Ca^{2+} influx and immune activation	- Reduced sneezing and nasal rubbing - Reduced epithelial cell proliferation, eosinophil infiltration and cytokine expression - Downregulated STIM1, ORAI1 and K_{CA}3.1 expression	95
ORAI1 expression silencing (short-hairpin RNA)	AR mice	ORAI1 is crucial for the Ca^{2+} intracellular increase necessary for immune cells activation. It is upregulated in allergic conditions	- Reduced NHR symptoms - Reduced inflammatory mediators in lavage serum	98
ORAI1 disruptors (2-APB)	AR mice	2-APB inhibits Ca^{2+} release–activated Ca^{2+} channels, as ORAI1, diminishing storage operated Ca^{2+} entry which mediates immune activation	- Reduction of NHR symptoms (rubbing, sneezing) - Reduction of eosinophils infiltration in nasal mucosa - Reduction of ORAI1 expression and inflammatory markers (IL4, ovalbumin-IgE)	87
ORAI1 disruptors (*Flors Magnoliae*)	AR mice	Fargesin, eudesmin and magnolin, three natural components of *F. Magnoliae* inhibit storage operated $Ca2^+$ entry mediated by ORAI1, reducing immune activation	- Reduced ORAI1-mediated Ca^{2+} current - Downregulation of immune response, such as mast cells degranulation and T-cell proliferation	85
ORAI1 disruptors (specific antibodies)	AR mice	Direct protein inhibition	- Reduction of NHR symptoms (rubbing, sneezing) - Reduction of eosinophils infiltration in nasal mucosa - Reduction of ORAI1 expression and inflammatory markers (ILs, histamine…)	86

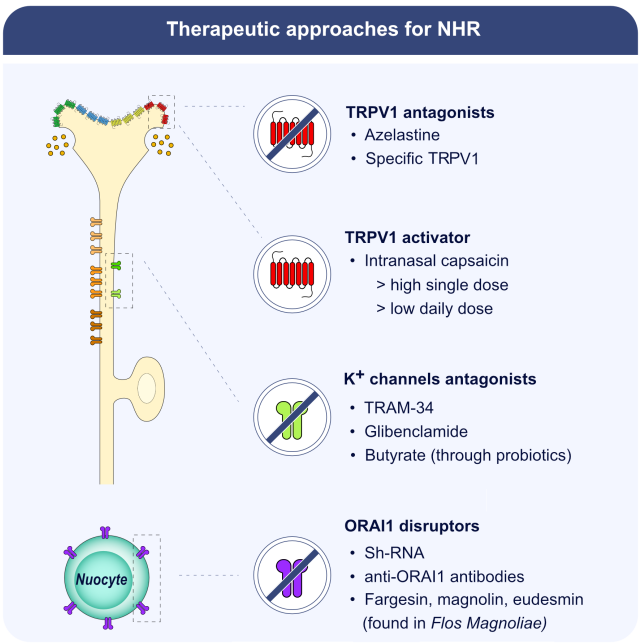

FIGURE 4 Therapeutic approaches to treat nasal hyperreactivity. Multiple molecular players implicated in NHR constitute potential targets for therapeutic approaches. These include TRPV1 antagonists, the TRPV1 agonist capsaicin (used to defunctionalize nociceptive nerve endings), K_V channel blockers and innovative antibody therapy against ORAI1. See Table 2 for more details

present with self-reported NHR. Also in asthma, a multicenter trial in different continents revealed the presence of NHR in 2/3 of patients with asthma,[105] with more than three triggers of NHR recognized in over 50% of asthma patients and with more NHR in the more severely affected asthma patients. Interestingly, bronchial hyperreactivity (BHR) was reported more frequently in asthma patients with NHR than in those without NHR, and with more BHR in the more severe asthma patients.[105] However, the neural pathways giving rise to NHR and BHR in asthma have not been studied in the context of upper and lower airway inflammation in the same asthma patient, nor any study has thus far focused on the effects of treating NHR on BHR and/or asthma. Given that a majority of patients with both rhinitis and rhinosinusitis remain uncontrolled with persistent NHR despite medical and surgical therapy,[106,107] better insight into the neurogenic pathways giving rise to NHR might lead to more disease control, less dissatisfaction and novel opportunities for care in CRS.

Along this review, we discussed several treatments for NHR and the conditions underlying it. These are summarized in Table 2 and Figure 4. It is notable that almost all NHR treatments (11 out of 12) have been studied in allergic rhinitis animal models or patients, and only one of them having been tested also in non-allergic rhinitis. Therefore, the generalization of the results of these treatments to all forms of NHR is not granted. Thus, further studies in other pathologies producing NHR should be performed to specifically design therapies for these patients, and to identify what mechanisms underlying NHR are specific or generalizable to all pathologies.

4 | CONCLUSIONS

Rhinitis, even at it's simplest forms, is determined by multiple interactions between the cell types forming the nasal mucosa, and by plethora of molecular players. The term NHR has emerged from clinical practice to highlight the sensory dimension of this pathology. Nonetheless, by its general character, this concept is apparently obsolete regarding current recommendations for the understanding of rhinitis in terms of defined endotypes. For a better comprehension of rhinitis pathophysiology and endotypes, it seems convenient to dissect NHR in at least three categories: deficient barrier function, hypersensitivity and hyperresponsiveness. To identify the pathological mechanisms operating in each patient, it would be ideal to develop clinical tests able to separately evaluate the function of each subsystem implicated. This seems critical in the case of idiopathic rhinitis, in which the dry cold air provocation test is currently the best tool to assess NHR. This contrasts with the fact that it remains unclear if hyperreactivity to cold is the only useful characteristic to determine the pathological mechanism in each patient. Moreover, the cold sensing mechanism of the human nasal mucosa is still unknown. Of the two principal candidates, TRPM8 and TRPA1, there is no description of the expression patterns, nor a characterization of their functional properties in nasal afferent innervation. In summary, although great progress has been made in the identification of potential molecular players, much remains to be done in the study of hyperreactivity pathophysiology in rhinitis.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization, E.V., L.V.G and K.T.; writing, E.V., L.V.G, P.W.H, J.G and K.T.; visualization, E.V. and M.D-M.; supervision, J.G. and K.T.; funding acquisition, E.V., P.W.H, J.G. and K.T. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Li F, Jiang H, Shen X, et al. Sneezing reflex is mediated by a peptidergic pathway from nose to brainstem. *Cell*. 2021;184(14):3762-3773 e3710.
2. Songu M, Cingi C. Sneeze reflex: facts and fiction. *Ther Adv Respir Dis*. 2009;3(3):131-141.
3. Gerth van Wijk RG, de Graaf-in 't Veld C, Garrelds IM. Nasal hyperreactivity. *Rhinology*. 1999;37(2):50-55.
4. Van Gerven L, Steelant B, Hellings PW. Nasal hyperreactivity in rhinitis: a diagnostic and therapeutic challenge. *Allergy*. 2018;73(9):1784-1791.
5. Backaert W, Steelant B, Jorissen M, et al. Self-reported nasal hyperreactivity is common in all chronic upper airway inflammatory phenotypes and not related to general well-being. *Allergy*. 2021;76(12):3806–3809.
6. Doulaptsi M, Steelant B, Prokopakis E, et al. Prevalence and impact of nasal hyperreactivity in chronic rhinosinusitis. *Allergy*. 2020;75(7):1768-1771.
7. Dordal MT, Lluch-Bernal M, Sanchez MC, et al. Allergen-specific nasal provocation testing: review by the rhinoconjunctivitis committee of the Spanish Society of Allergy and Clinical Immunology. *J Investig Allergol Clin Immunol*. 2011;21(1):1-12. quiz follow 12.
8. Gosepath J, Amedee RG, Mann WJ. Nasal provocation testing as an international standard for evaluation of allergic and nonallergic rhinitis. *Laryngoscope*. 2005;115(3):512-516.
9. Lluch-Bernal M, Dordal MT, Anton E, et al. Nasal hyperreactivity: nonspecific nasal provocation tests. Review by the Rhinoconjunctivitis Committee of the Spanish Society of Allergy and Clinical Immunology. *J Investig Allergol Clin Immunol*. 2015;25(6):396-407.
10. Salzano FA. Specific nasal provocation test with powder allergen. *Allergy*. 1997;52(33 Suppl):32-35.
11. De Greve G, Hellings PW, Fokkens WJ, Pugin B, Steelant B, Seys SF. Endotype-driven treatment in chronic upper airway diseases. *Clin Transl Allergy*. 2017;7:22.
12. Bernstein JA, Singh U. Neural abnormalities in nonallergic rhinitis. *Curr Allergy Asthma Rep*. 2015;15(4):18.
13. Rubel EW, Bernstein JM. The role of autonomic nervous system and inflammatory mediators in nasal hyperreactivity: a review. *Otolaryngol Head Neck Surg*. 1991;105(4):596-607.
14. Holtzman MJ, Look DC, Sampath D, Castro M, Koga T, Walter MJ. Control of epithelial immune-response genes and implications for airway immunity and inflammation. *Proc Assoc Am Physicians*. 1998;110(1):1-11.
15. Undem BJ, Taylor-Clark T. Mechanisms underlying the neuronal-based symptoms of allergy. *J Allergy Clin Immunol*. 2014;133(6):1521-1534.
16. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. *J Allergy Clin Immunol*. 2020;145(6):1499-1509.
17. Steelant B, Seys SF, Boeckxstaens G, Akdis CA, Ceuppens JL, Hellings PW. Restoring airway epithelial barrier dysfunction: a new therapeutic challenge in allergic airway disease. *Rhinology*. 2016;54(3):195-205.
18. Miller JD. The role of dust mites in allergy. *Clin Rev Allergy Immunol*. 2019;57(3):312-329.
19. Rogers GA, Den Beste K, Parkos CA, Nusrat A, Delgaudio JM, Wise SK. Epithelial tight junction alterations in nasal polyposis. *Int Forum Allergy Rhinol*. 2011;1(1):50-54.
20. Runswick S, Mitchell T, Davies P, Robinson C, Garrod DR. Pollen proteolytic enzymes degrade tight junctions. *Respirology*. 2007;12(6):834-842.
21. Tatsuta M, Kan-o K, Ishii Y, et al. Effects of cigarette smoke on barrier function and tight junction proteins in the bronchial epithelium: protective role of cathelicidin LL-37. *Respir Res*. 2019;20(1):251.
22. Zhao R, Guo Z, Zhang R, et al. Nasal epithelial barrier disruption by particulate matter </=2.5 mum via tight junction protein degradation. *J Appl Toxicol*. 2018;38(5):678-687.
23. Fukuoka A, Yoshimoto T. Barrier dysfunction in the nasal allergy. *Allergol Int*. 2018;67(1):18-23.
24. Steelant B, Farré R, Wawrzyniak P, et al. Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. *J Allergy Clin Immunol*. 2016;137(4):1043-1053 e1045.
25. Steelant B, Seys SF, Van Gerven L, et al. Histamine and T helper cytokine-driven epithelial barrier dysfunction in allergic rhinitis. *J Allergy Clin Immunol*. 2018;141(3):951-963.e8.
26. Feliciangeli S, Chatelain FC, Bichet D, Lesage F. The family of K2P channels: salient structural and functional properties. *J Physiol*. 2015;593(12):2587-2603.
27. Jiang J, Liu J-Q, Li J, et al. Trek1 contributes to maintaining nasal epithelial barrier integrity. *Sci Rep*. 2015;5:9191.
28. Wang Y, Lv L, Zang H, et al. Regulation of Trek1 expression in nasal mucosa with allergic rhinitis by specific immunotherapy. *Cell Biochem Funct*. 2015;33(1):23-28.
29. Wittekindt OH, Dietl P. Aquaporins in the lung. *Pflugers Arch*. 2019;471(4):519-532.
30. Song Y, Verkman AS. Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J Biol Chem*. 2001;276(44):41288-41292.
31. Frauenfelder C, Woods C, Hussey D, Ooi E, Klebe S, Carney AS. Aquaporin expression profiles in normal sinonasal mucosa and chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2014;4(11):901-908.
32. Shikani AH, Sidhaye VK, Basaraba RJ, et al. Mucosal expression of aquaporin 5 and epithelial barrier proteins in chronic rhinosinusitis with and without nasal polyps. *Am J Otolaryngol*. 2014;35(3):377-383.
33. Chang YL, Lin CS, Wang HW, Jian KR, Liu SC. Chlorpheniramine attenuates histamine-mediated aquaporin 5 downregulation in human nasal epithelial cells via suppression of NF-kappaB activation. *Int J Med Sci*. 2017;14(12):1268-1275.
34. Sleigh MA. Adaptations of ciliary systems for the propulsion of water and mucus. *Comp Biochem Physiol A Comp Physiol*. 1989;94(2):359-364.
35. Shimizu T. Mucus, goblet cell, submucosal gland. In: Önerci TM, ed. *Nasal Physiology and Pathophysiology of Nasal Disorders*. Springer-Verlag; 2013:1-14.
36. Gohy S, Hupin C, Ladjemi MZ, Hox V, Pilette C. Key role of the epithelium in chronic upper airways diseases. *Clin Exp Allergy*. 2020;50(2):135-146.
37. Allergy CMK. Histamine and Antihistamines. In: Hattori Y, Seifert R, eds. *Histamine and Histamine Receptors in Health and Disease. Handbook of Experimental Pharmacology*. Vol 241. Springer; 2016:321-331.
38. Bhargave G, Woodworth BA, Xiong G, Wolfe SG, Antunes MB, Cohen NA. Transient receptor potential vanilloid type 4 channel expression in chronic rhinosinusitis. *Am J Rhinol*. 2008;22(1):7-12.
39. Andrade YN, Fernandes J, Vázquez E, et al. TRPV4 channel is involved in the coupling of fluid viscosity changes to epithelial ciliary activity. *J Cell Biol*. 2005;168(6):869-874.
40. Rollin M, Seymour K, Hariri M, Harcourt J. Rhinosinusitis, symptomatology & absence of polyposis in children with primary ciliary dyskinesia. *Rhinology*. 2009;47(1):75-78.
41. Wynne M, Atkinson C, Schlosser RJ, Mulligan JK. Contribution of epithelial cell dysfunction to the pathogenesis of chronic rhinosinusitis with nasal polyps. *Am J Rhinol Allergy*. 2019;33(6):782-790.
42. Guan W-J, Peng Y, Zi X-X, et al. Motile ciliary disorders in chronic airway inflammatory diseases: critical target for interventions. *Curr Allergy Asthma Rep*. 2018;18(9):48.
43. Steelant B, Hox V, Hellings PW, Bullens DM, Seys SF. Exercise and sinonasal disease. *Immunol Allergy Clin North Am*. 2018;38(2):259-269.

44. Sanchez A, Alvarez JL, Demydenko K, et al. Silica nanoparticles inhibit the cation channel TRPV4 in airway epithelial cells. *Part Fibre Toxicol*. 2017;14(1):43.
45. Workman AD, Carey RM, Chen B, et al. CALHM1-mediated ATP release and ciliary beat frequency modulation in nasal epithelial cells. *Sci Rep*. 2017;7(1):6687.
46. Kim HJ, Seo YH, An S, Jo A, Kwon IC, Kim S. Chemiluminescence imaging of Duox2-derived hydrogen peroxide for longitudinal visualization of biological response to viral infection in nasal mucosa. *Theranostics*. 2018;8(7):1798-1807.
47. Talavera K, Startek JB, Alvarez-Collazo J, et al. Mammalian transient receptor potential TRPA1 channels: from structure to disease. *Physiol Rev*. 2020;100(2):725-803.
48. Alpizar YA, Boonen B, Sanchez A, et al. TRPV4 activation triggers protective responses to bacterial lipopolysaccharides in airway epithelial cells. *Nat Commun*. 2017;8(1):1059.
49. Martel J, Ko YF, Young JD, Ojcius DM. Could nasal nitric oxide help to mitigate the severity of COVID-19? *Microbes Infect*. 2020;22(4–5):168-171.
50. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than just signaling the alarm. *J Clin Invest*. 2019;129(4):1441-1451.
51. Xu M, Yang W, Wang X, Nayak DK. Lung secretoglobin Scgb1a1 influences alveolar macrophage-mediated inflammation and immunity. *Front Immunol*. 2020;11:584310.
52. Flockerzi V, Nilius B. TRPs: truly remarkable proteins. *Handb Exp Pharmacol*. 2014;222:1-12.
53. Nilius B, Flockerzi V. What do we really know and what do we need to know: some controversies, perspectives, and surprises. *Handb Exp Pharmacol*. 2014;223:1239-1280.
54. Viana F. TRPA1 channels: molecular sentinels of cellular stress and tissue damage. *J Physiol*. 2016;594(15):4151-4169.
55. Matsuda M, Huh Y, Ji RR. Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. *J Anesth*. 2019;33(1):131-139.
56. Cavaliere C, Masieri S, Cavaliere F. Therapeutic applications of capsaicin in upper airways. *Curr Drug Targets*. 2018;19(10):1166-1176.
57. Van Rijswijk JB, Boeke EL, Keizer JM, Mulder PG, Blom HM, Fokkens WJ. Intranasal capsaicin reduces nasal hyperreactivity in idiopathic rhinitis: a double-blind randomized application regimen study. *Allergy*. 2003;58(8):754-761.
58. Hellings PW, Klimek L, Cingi C, et al. Non-allergic rhinitis: position paper of the European Academy of Allergy and Clinical Immunology. *Allergy*. 2017;72(11):1657-1665.
59. Van Gerven L, Alpizar YA, Wouters MM, et al. Capsaicin treatment reduces nasal hyperreactivity and transient receptor potential cation channel subfamily V, receptor 1 (TRPV1) overexpression in patients with idiopathic rhinitis. *J Allergy Clin Immunol*. 2014;133(5):1332–1339, 1339 e1331–1333.
60. Jordt S-E, Bautista DM, Chuang H-H, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature*. 2004;427(6971):260-265.
61. Alpizar YA, Boonen B, Gees M, et al. Allyl isothiocyanate sensitizes TRPV1 to heat stimulation. *Pflugers Arch*. 2014;466(3):507-515.
62. Everaerts W, Gees M, Alpizar YA, et al. The capsaicin receptor TRPV1 is a crucial mediator of the noxious effects of mustard oil. *Curr Biol*. 2011;21(4):316-321.
63. Gees M, Alpizar YA, Boonen B, et al. Mechanisms of transient receptor potential vanilloid 1 activation and sensitization by allyl isothiocyanate. *Mol Pharmacol*. 2013;84(3):325-334.
64. Janssens A, Gees M, Toth BI, et al. Definition of two agonist types at the mammalian cold-activated channel TRPM8. *Elife*. 2016;5. doi:10.7554/eLife.17240
65. Van Gerven L, Alpizar YA, Steelant B, et al. Enhanced chemosensory sensitivity in patients with idiopathic rhinitis and its reversal by nasal capsaicin treatment. *J Allergy Clin Immunol*. 2017;140(2):437-446 e432.
66. Van Gerven L, Steelant B, Cools L, et al. Low-dose capsaicin (0.01 mM) nasal spray is equally effective as the current standard treatment for idiopathic rhinitis: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol*. 2021;147(1):397-400.
67. Alenmyr L, Hogestatt ED, Zygmunt PM, Greiff L. TRPV1-mediated itch in seasonal allergic rhinitis. *Allergy*. 2009;64(5):807-810.
68. Gerth Van Wijk R, Terreehorst IT, Mulder PG, Garrelds IM, Blom HM, Popering S. Intranasal capsaicin is lacking therapeutic effect in perennial allergic rhinitis to house dust mite. A placebo-controlled study. *Clin Exp Allergy*. 2000;30(12):1792-1798.
69. Alenmyr L, Greiff L, Andersson M, Sterner O, Zygmunt PM, Hogestatt ED. Effect of mucosal TRPV1 inhibition in allergic rhinitis. *Basic Clin Pharmacol Toxicol*. 2012;110(3):264-268.
70. Bareille P, Murdoch RD, Denyer J, et al. The effects of a TRPV1 antagonist, SB-705498, in the treatment of seasonal allergic rhinitis. *Int J Clin Pharmacol Ther*. 2013;51(7):576-584.
71. Kortekaas Krohn I, Callebaut I, Alpizar YA, et al. MP29-02 reduces nasal hyperreactivity and nasal mediators in patients with house dust mite-allergic rhinitis. *Allergy*. 2018;73(5):1084-1093.
72. Van Gerven L, Boeckxstaens G, Jorissen M, Fokkens W, Hellings PW. Short-time cold dry air exposure: a useful diagnostic tool for nasal hyperresponsiveness. *Laryngoscope*. 2012;122(12):2615-2620.
73. Acosta CM, Luna C, Quirce S, Belmonte C, Gallar J. Changes in sensory activity of ocular surface sensory nerves during allergic keratoconjunctivitis. *Pain*. 2013;154(11):2353-2362.
74. Moparthi L, Kichko TI, Eberhardt M, et al. Human TRPA1 is a heat sensor displaying intrinsic U-shaped thermosensitivity. *Sci Rep*. 2016;6:28763.
75. Keh SM, Facer P, Yehia A, Sandhu G, Saleh HA, Anand P. The menthol and cold sensation receptor TRPM8 in normal human nasal mucosa and rhinitis. *Rhinology*. 2011;49(4):453-457.
76. Liu SC, Lu HH, Fan HC, et al. The identification of the TRPM8 channel on primary culture of human nasal epithelial cells and its response to cooling. *Medicine*. 2017;96(31):e7640.
77. Sozansky J, Houser SM. Pathophysiology of empty nose syndrome. *Laryngoscope*. 2015;125(1):70-74.
78. Hirata H, Dallacasagrande V, Mizerska K, Ivakhnitskaia E, Rosenblatt MI. Ambient air currents activate corneal nerves during ocular desiccation in rats: simultaneous recordings of neural activity and corneal temperature. *Invest Ophthalmol Vis Sci*. 2018;59(10):4031-4043.
79. Parra A, Madrid R, Echevarria D, et al. Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. *Nat Med*. 2010;16(12):1396-1399.
80. Nocchi L, Daly DM, Chapple C, Grundy D. Induction of oxidative stress causes functional alterations in mouse urothelium via a TRPM8-mediated mechanism: implications for aging. *Aging Cell*. 2014;13(3):540-550.
81. Tokunaga T, Ninomiya T, Kato Y, et al. The significant expression of TRPV3 in nasal polyps of eosinophilic chronic rhinosinusitis. *Allergol Int*. 2017;66(4):610-616.
82. Alonso-Carbajo L, Alpizar YA, Startek JB, Lopez-Lopez JR, Perez-Garcia MT, Talavera K. Activation of the cation channel TRPM3 in perivascular nerves induces vasodilation of resistance arteries. *J Mol Cell Cardiol*. 2019;129:219-230.
83. Fukuoka T, Kobayashi K, Yamanaka H, Obata K, Dai Y, Noguchi K. Comparative study of the distribution of the alpha-subunits of voltage-gated sodium channels in normal and axotomized rat dorsal root ganglion neurons. *J Comp Neurol*. 2008;510(2):188-206.
84. Bennett DL, Clark AJ, Huang J, Waxman SG, Dib-Hajj SD. The role of voltage-gated sodium channels in pain signaling. *Physiol Rev*. 2019;99(2):1079-1151.

85. Keh SM, Facer P, Simpson KD, Sandhu G, Saleh HA, Anand P. Increased nerve fiber expression of sensory sodium channels $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ in rhinitis. *Laryngoscope*. 2008;118(4):573-579.
86. Bonnet C, Hao J, Osorio N, et al. Maladaptive activation of $Na_V1.9$ channels by nitric oxide causes triptan-induced medication overuse headache. *Nat Commun*. 2019;10(1):4253.
87. Kapoor R, Li YG, Smith KJ. Slow sodium-dependent potential oscillations contribute to ectopic firing in mammalian demyelinated axons. *Brain*. 1997;120(Pt 4):647-652.
88. Puil E, Spigelman I. Electrophysiological responses of trigeminal root ganglion neurons in vitro. *Neuroscience*. 1988;24(2):635-646.
89. Velasco E, Alvarez JL, Meseguer VM, Gallar J, Talavera K. Membrane potential instabilities in sensory neurons: mechanisms and pathophysiological relevance. *Pain*. 2021;163(1):64–74.
90. Johnston J, Forsythe ID, Kopp-Scheinpflug C. Going native: voltage-gated potassium channels controlling neuronal excitability. *J Physiol*. 2010;588(Pt 17):3187-3200.
91. Cook DI, Young JA. Effect of K^+ channels in the apical plasma membrane on epithelial secretion based on secondary active Cl^- transport. *J Membr Biol*. 1989;110(2):139-146.
92. Bardou O, Trinh NT, Brochiero E. Molecular diversity and function of K^+ channels in airway and alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2009;296(2):L145-L155.
93. Nomori H, Watanabe S, Nakajima T, Shimosato Y, Kameya T. Histiocytes in nasopharyngeal carcinoma in relation to prognosis. *Cancer*. 1986;57(1):100-105.
94. Sakai H, Enzaka J, Sakai-Oshita M, Uto F, Chiba Y, Misawa M. Involvement of K^+ channels in the augmented nasal venous responsiveness to nitric oxide in rat model of allergic rhinitis. *Microvasc Res*. 2011;81(1):129-134.
95. Lin H, Zheng C, Li J, Yang C, Hu L. Ca^{2+} -activated K^+ channel-3.1 blocker TRAM-34 alleviates murine allergic rhinitis. *Int Immunopharmacol*. 2014;23(2):642-648.
96. Yu Z-H, Xu J-R, Wang Y-X, et al. Targeted inhibition of KCa3.1 channel attenuates airway inflammation and remodeling in allergic asthma. *Am J Respir Cell Mol Biol*. 2013;48(6):685-693.
97. Lin L, Zheng C, Zhang L, Da C, Zhao K. Up-regulation of Orai1 in murine allergic rhinitis. *Histochem Cell Biol*. 2010;134(1):93-102.
98. Wang Y, Lin L, Zheng C. Downregulation of Orai1 expression in the airway alleviates murine allergic rhinitis. *Exp Mol Med*. 2012;44(3):177-190.
99. Lin L, Wei J, Chen Z, Tang X, Dai F, Sun G. Intervention of Orai1 influences the response of nuocytes from allergic rhinitis mice to IL-33. *Ann Otol Rhinol Laryngol*. 2019;128(9):838-847.
100. Hong PTL, Kim HJ, Kim WK, Nam JH. Flos magnoliae constituent fargesin has an anti-allergic effect via ORAI1 channel inhibition. *Korean J Physiol Pharmacol*. 2021;25(3):251-258.
101. Lin L, Dai F, Sun T. The Orai1 antibody treatment for a mouse model of allergic rhinitis. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2016;51(1):43-49.
102. Lin L, Zheng C, Zhang L, Da C, Zhao K. 2-Aminoethoxydiphenyl borate administration into the nostril alleviates murine allergic rhinitis. *Am J Otolaryngol*. 2011;32(4):318-328.
103. Li H, Wen W, Cheng L, et al. Expression of chloride channel protein CLC-3 in patients with allergic rhinitis: effect of topical corticosteroid treatment. *Arch Otolaryngol Head Neck Surg*. 2008;134(3):301-305.
104. Khoo SG, Al-Alawi M, Walsh MT, et al. Eosinophil peroxidase induces the expression and function of acid-sensing ion channel-3 in allergic rhinitis: in vitro evidence in cultured epithelial cells. *Clin Exp Allergy*. 2012;42(7):1028-1039.
105. Feijen J, Seys SF, Steelant B, et al. Prevalence and triggers of self-reported nasal hyperreactivity in adults with asthma. *World Allergy Organ J*. 2020;13(6):100132.
106. Van Bulck P, Cools L, Soumya MS, et al. A multicenter real-life study on the multiple reasons for uncontrolled allergic rhinitis. *Int Forum Allergy Rhinol*. 2021;11(10):1452-1460.
107. Hellings PW, Fokkens WJ, Akdis C, et al. Uncontrolled allergic rhinitis and chronic rhinosinusitis: where do we stand today? *Allergy*. 2013;68(1):1-7.

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