Cough Reflex Testing With Inhaled Capsaicin and TRPV1 Activation in Asthma and Comorbid Conditions

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Abstract

A high parasympathetic tone leading to bronchoconstriction and neurogenic inflammation is thought to have a major role in the pathogenesis of asthma. Transient receptor potential vanilloid 1 (TRPV1) is the hub of almost all neuronal inflammatory signaling pathways. A critical determinant of neurogenic inflammation, TRPV1 functions as a sensor for detecting irritants in the lung by transmitting noxious stimuli to the central nervous system and inducing the release of a variety of proinflammatory neuropeptides at the peripheral terminals.
Challenge with inhaled capsaicin, an exogenous agonist of TRPV1, has been used to measure the sensitivity of the cough reflex. However, inhalation of capsaicin is also associated with parasympathetic bronchoconstriction, mucus hypersecretion, vasodilatation, and the sensation of dyspnea. Therefore, inhaled capsaicin challenge is expected to have other potential applications in asthma and comorbid conditions, such as rhinitis and gastroesophageal reflux disease, both of which produce cough. Capsaicin challenge has established itself as a useful objective method for evaluating airway hypersensitivity; however, it is potentially valuable in many other situations, which will be reviewed in this paper.

Key words: Asthma. Airway sensory hyperreactivity. Capsaicin. Cough. Gastroesophageal reflux. Rhinitis. Transient receptor potential vanilloid 1.

Resumen

Se ha constatado en el asma la presencia de un tono parasimpático elevado que induce broncoconstricción e inflamación neurogénica en la vía aérea.
Estas anomalías podrían jugar un papel primordial en la patogenia de la enfermedad. El receptor de vanilloid 1 (TRPV1) centraliza el control de todas las señales neurogénicas proinflamatorias. Es un receptor pulmonar sensible al daño y transmite toda la información del mismo al sistema nervioso central induciendo la liberación de diversos neuropépticos proinflamatorios a nivel de las terminaciones periféricas.
La provocación con capsaicina inhalada, un agonista exógeno del receptor TRPV1, ha sido extensamente utilizada para cuantificar el reflejo de la tos. Sin embargo, su inhalación también se asocia con broncoconstricción parasimpática, hipersecreción mucosa, vasodilatación y disnea. Por tanto, es esperable que esta técnica podría potencialmente también tener otras aplicaciones en el estudio del asma y sus co-morbilidades causantes de tos, como son la rinitis o el reflujo gastroesofágico. Por tanto, además del papel en el estudio de esta reactividad sensorial de la vía aérea, se revisarán también otras posibles aplicaciones.

Palabras clave: Asma. Reactividad sensorial de la vía aérea. Capsaicina. Tos. Reflujo gastroesofágico. Rinitis. Receptor de vanilloid 1.

Introduction

Asthma is a clinical syndrome comprising intermittent respiratory symptoms triggered by viral infections, environmental allergens, and other stimuli and characterized by nonspecific airway hyperresponsiveness and inflammation [1]. The latter is a multicellular process involving airway epithelium, eosinophils, neutrophils, lymphocytes, and mast cells [2]. Through vascular adhesion and control of permeability, the endothelium mediates infiltration of immune cells and the formation of edema in the lungs [3]. Bronchoconstriction is largely dependent on airway smooth muscle cells; in addition to intense inflammatory and immunologic cell infiltration, it is generally accepted that airway vagal sensory neurons partially account for bronchoconstriction [4-6]. Chemical stimulation of vagal sensory fibers by irritants reaching the lower airways can trigger tracheal and bronchial constriction, bronchospasm, mucus secretion, and neurogenic inflammation [7].

Neurogenic inflammation in the lung involves an increase in vascular permeability, extravasation of plasma and leukocytes, mucus hypersecretion, airway constriction, and release of additional inflammatory mediators [5,8]. The neurogenic inflammatory pathway is associated with release and action of neuropeptides, including tachykinins or calcitonin gene–related peptide (CGRP) from primary sensory nerve terminals as a response of sensory neurons to inflammatory mediators and noxious stimuli [5,8]. Tachykinins are potent neurogenic mediators of a number of functions in the airways [9]. In the human airway, substance P and neurokinin (NK) A are the predominant neuropeptides released by the nonadrenergic-noncholinergic nervous system; they act mainly through their NK1 and NK2 receptors in response to mechanical, thermal, chemical, and inflammatory stimuli [10,11]. NK receptor antagonists decrease airway responsiveness and may improve lung function, although their effects on airway inflammation and asthma symptoms are poorly studied [10].

Transient receptor potential vanilloid 1 (TRPV1), a member of the TRP channel superfamily, is the hub of almost all neuronal inflammatory signaling pathways [12]. It is expressed in primary sensory neurons, pulmonary smooth muscle cells, bronchial and tracheal epithelial cells, and dendritic cells in the lung. In addition, it is often colocalized with sensory neuropeptides in the same axon of a primary neuron [12]. Vanilloid-sensitive neurons transmit noxious stimuli to the central nervous system (afferent function), whereas the peripheral terminals of these neurons are sites of release for a variety of proinflammatory neuropeptides (efferent function). TRPV1 seems to play a particularly important role in nonmyelinated tachykinin-containing C fibers, toward which all major neuronal inflammatory pathways converge, thus increasing C-fiber excitability during airway inflammation [7]. Their activation by noxious temperature, changes in membrane potential, low pH, and mechanical or osmotic stress leads to release of neuropeptides with potent effects on airway smooth muscle tone, airway secretion, edema, and the chemoattraction of inflammatory and immune cells [8]. Moreover, TRPV1 channels can be activated by various chemical agonists, including endogenous signal transduction molecules (eg, phosphatidylinositol phosphates), arachidonic acid metabolites, and specific exogenous molecules (eg, capsaicin) [8] (Figure 1).

The mechanism of action of capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide), the pungent ingredient of red peppers, is triggered by stimulation of specific neuronal TRPV1 receptors [13], which in turn produces a concentration-dependent increase in cell membrane permeability to ions such as calcium and sodium, thus facilitating neural conduction. Activation of capsaicin is common to reflexes of bronchoconstriction, bradycardia, pain, and hypotension, although it is modulated by different cholinergic or neuroinflammatory interactions [14]. Inhalation of capsaicin has been used as a challenge to measure the sensitivity of the cough reflex [15]. As this compound is a potent activator of TRPV1, inhalation leads to activation of airway C fibers, which is associated with parasympathetic bronchoconstriction, mucus hypersecretion, vasodilatation, cough, and sensation of dyspnea [16]. Moreover, administration of intranasal capsaicin by nebulizer causes watery rhinorrhea, sneezing, and nasal burning [17]. Therefore, challenge with inhaled capsaicin could have a role in the evaluation and follow-up of asthma and related cough-inducing comorbid conditions, such as rhinitis and gastroesophageal reflux disease (GERD). The aims of this study were as follows: first, to briefly describe the methodology of capsaicin challenge and TRPV1 activation; and second, to review the feasibility and safety of capsaicin challenge in asthma and comorbid conditions.

Inhaled Capsaicin Challenge

The procedure for inhaled capsaicin challenge should be standardized to facilitate universal interpretation and data comparison between different laboratories [15]. In the absence of published contraindications to capsaicin challenge and based on our experience, we suggest following the guidelines used for bronchial challenge with methacholine. Therefore, it seems wise to carry out spirometry before starting the capsaicin challenge. Even if no clinically significant bronchoconstriction is observed as a response to inhalation of capsaicin in healthy volunteers or asthmatics [18,19], in the interests of safety, it is advisable to repeat spirometry at the end of the challenge and to have bronchodilator therapy readily available [15].

Capsaicin in powder is dissolved in 1 mL of polyoxyethylene sorbitan (Tween 80) and 1 mL of ethanol before being mixed with 8 mL of normal saline to make a stock solution of 0.01 M, which is stored at –30°C [15,20]. Without the detergent Tween 80, a cloudy rather than clear solution results [15]. Dilutions of this solution are performed with physiological saline to obtain serial doubling concentrations ranging from 0.49 μM to 500 μM [15,21] (Figure 2). Until recently, it was unclear how often fresh dilutions from the stock solution should be prepared, and the recommended approach involved a fresh preparation on each day of testing. However, it was later concluded that capsaicin solutions of ≥4 μM are stable for 1 year if stored at 4ºC and protected from light [22].

Capsaicin is delivered in serial doubling concentrations, starting with normal saline inhalation, based on either single-dose or dose-response methods [23]. A recently published comparison of tidal breathing and dosimeter methods showed

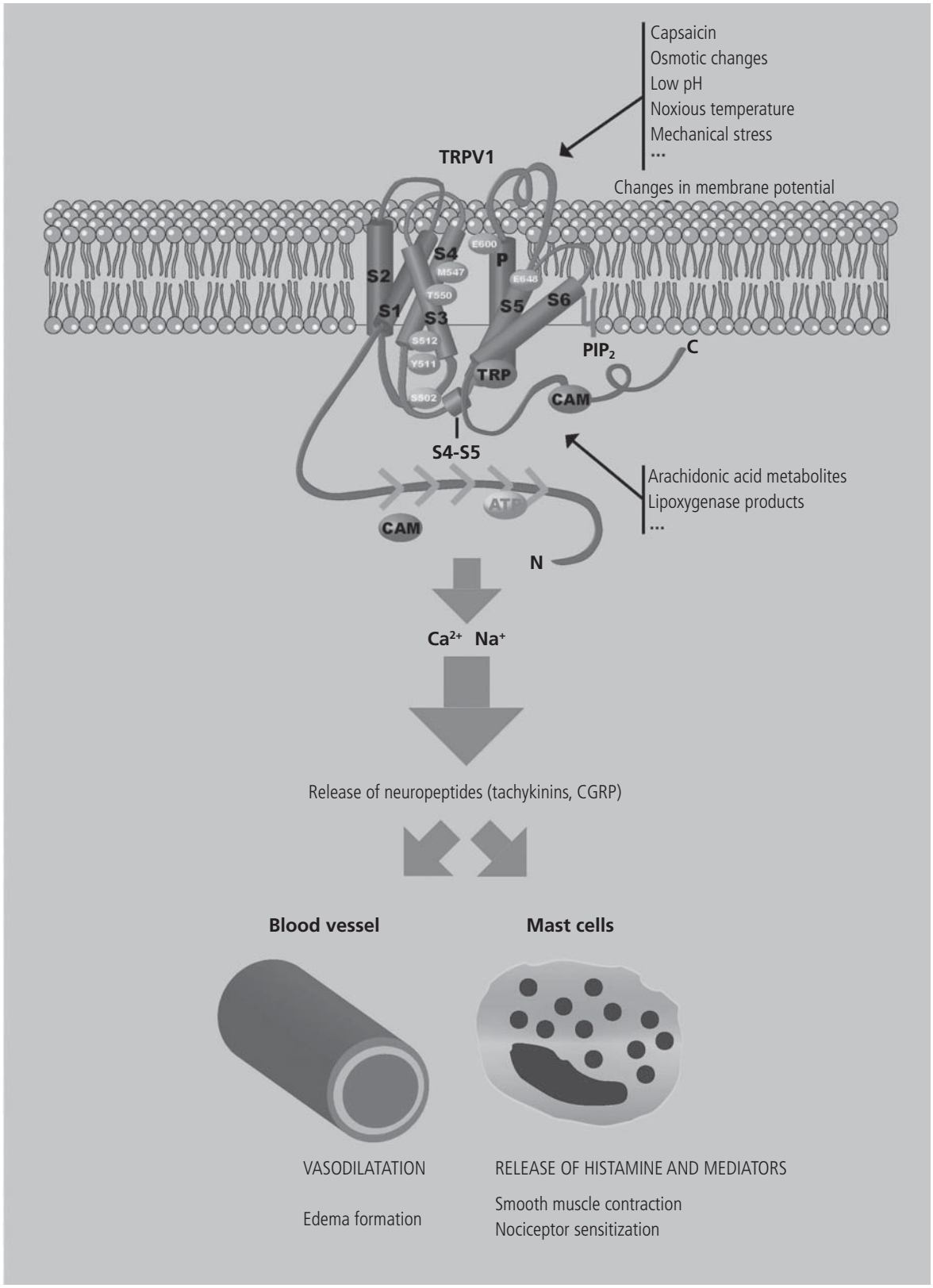

Figure 1. Simplified summary of activation of TRPV1 (transient receptor potential vanilloid 1), including endogenous and exogenous agonists, as well as the effects of neuropeptide release and neurogenic inflammation. ATP indicates adenosine 5′ triphosphate; CAM, cell adhesion molecule; CGRP calcitonin gene–related peptide.

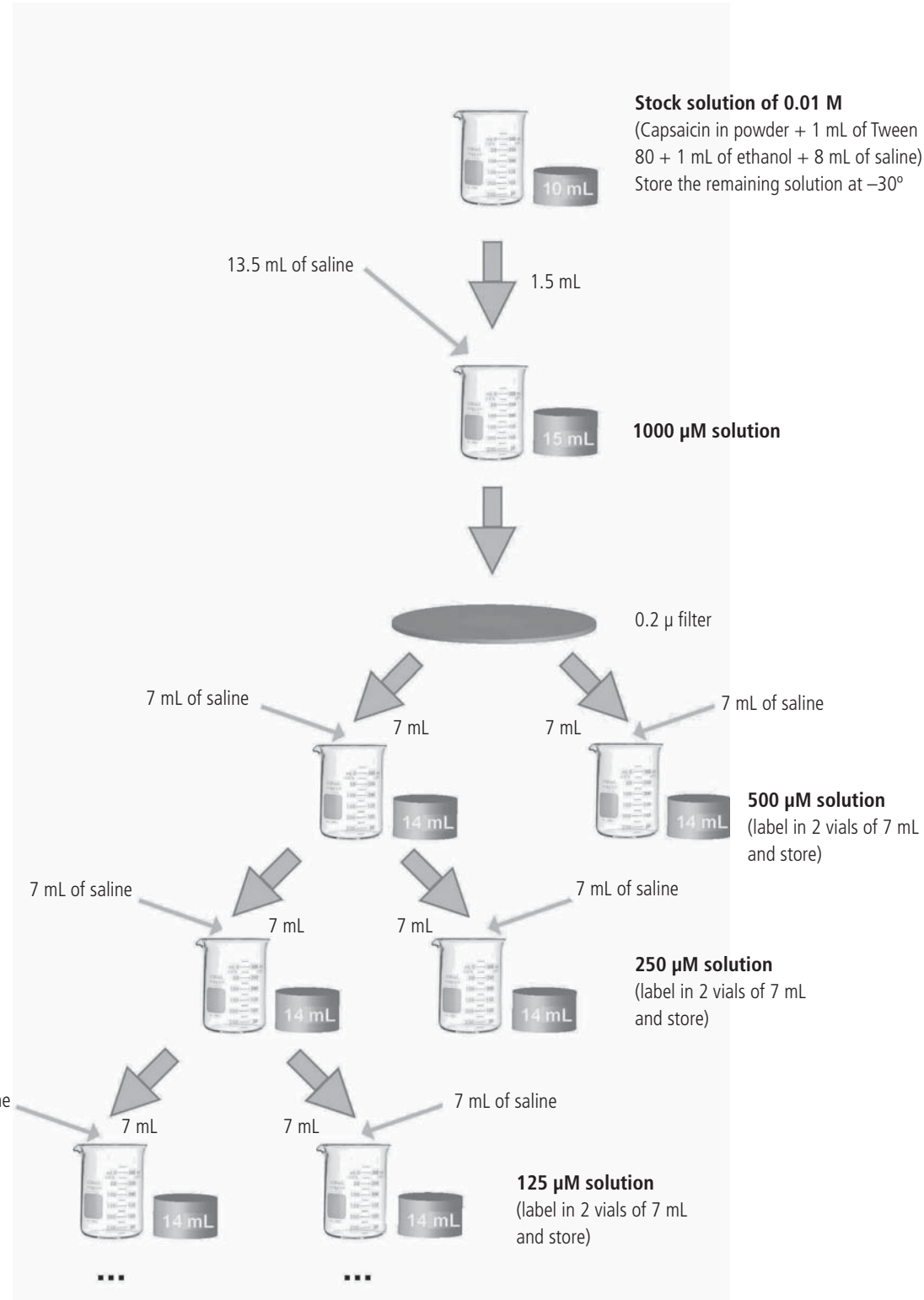

Figure 2. Preparation of capsaicin solutions and sequential dilutions with different concentrations. The same steps are followed until the 0.49-µM solution is reached.

both to be reproducible, with good agreement between them [24]. However, the single-breath concentration-response method using a flow-limited dosimeter is the approach recommended for most experimental protocols [15]. The method consists of a single concentration of capsaicin with incremental concentrations administered via a dosimeter-controlled nebulizer, each over a fixed period of time. The flow rate needs to be constant, and the exact output of the nebulizer must be determined in order to set the output per inhalation [15]. Our experience is based mainly on the single-breath method. We deliver aerosol through a breath-activated nebulizer controlled by a dosimeter (Optineb, Air Liquide) set to nebulize for 0.9 seconds [21,25]. Three milliliters of each solution (starting with the lower concentration and progressively increasing) is placed in the nebulizer and participants are asked to inhale once deeply over 2 seconds. Time between administrations is not completely standardized; however, it must remain unchanged for a specific challenge and be identified in the methods section when articles are published. Ranges are usually between 15 and 60 seconds in some recommendations and up to 2 minutes in others [21,25] (Figure 3).

Participants are asked to cough freely and as much as they need; they are specifically instructed not to talk immediately after inhalation, since this may potentially suppress cough. They should not be told that the endpoint of the study is induction of a specific number of coughs [15]. This strategy, together with the inhalation of randomly interspersed saline solution [15,26-28], may reduce the effects of voluntary suppression or conditioned responses in participants who would otherwise be anticipating progressively higher concentrations of tussive agent.

The cough response to each dose of aerosol is immediate and brief when the single-breath administration method is used. Therefore, only coughs occurring within 15 to 30 seconds of capsaicin delivery should be recorded [15,21,23,25,29]. The concentrations of the solutions inducing 2 coughs (C2) and 5 coughs (C5) are registered (Figure 3). The test is finished at C5 or when the maximal dose (500 μM) is attained. Despite the fact that a small subgroup of individuals with relatively high cough thresholds may not be able to achieve C5 even when the 500-μM solution is used, the inhalation of higher concentrations of capsaicin is precluded by a strong burning sensation in the upper airway and should therefore be avoided. Transient throat irritation is the only reported adverse event associated with inhaled capsaicin challenge testing in humans. A review of 20 years' clinical experience, including 122 published studies describing 4833 individuals (4374 adults and 459 children, including healthy volunteers and patients with

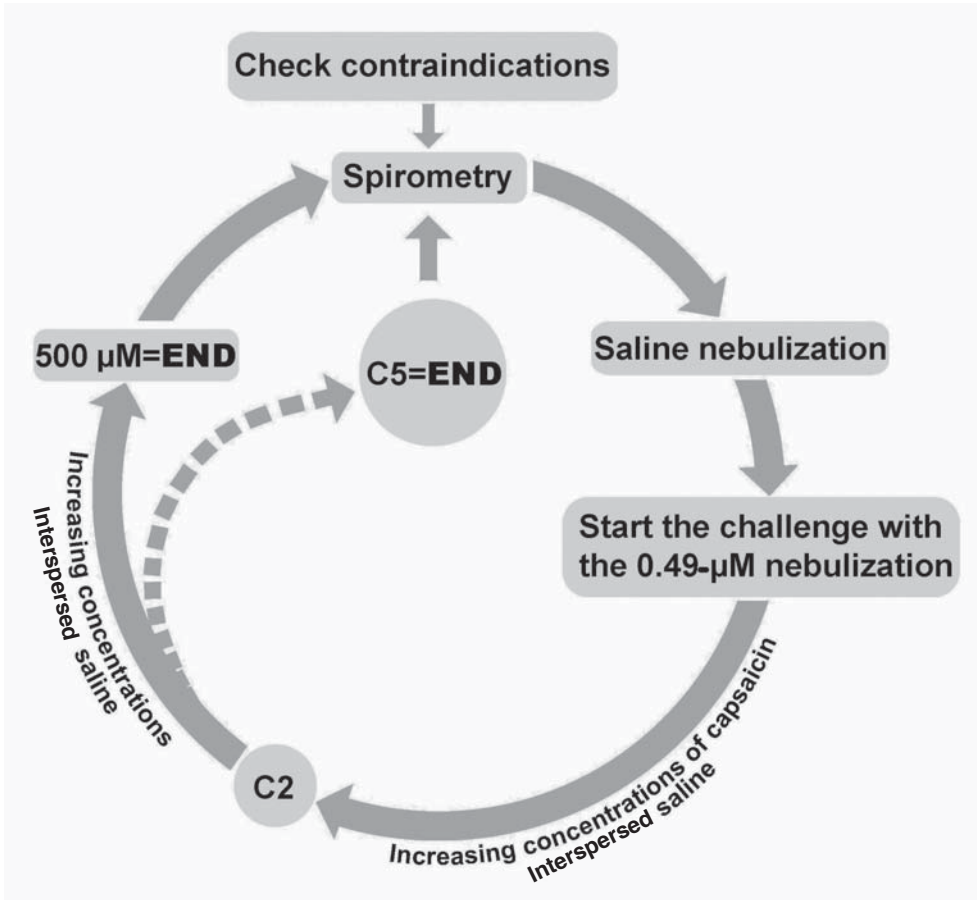

Figure 3. Summary of capsaicin challenge technique. C2 indicates 2 coughs; C5, 5 coughs.

asthma, chronic obstructive pulmonary disease, pathologic cough, and other respiratory conditions) undergoing capsaicin challenge failed to uncover a single severe adverse event [30]. Capsaicin challenge is therefore considered a very safe method.

Given the considerable variation in cough reflex sensitivity within the healthy population, isolated measurements of capsaicin sensitivity have not shown intrinsic significance to date [15]. This means that even if C2 and C5 differ between participants, they cannot be directly predictive of pathophysiology. Moreover, with currently available data, it is not possible to infer that a given participant is outside the normal range. This is probably the reason for some of the discrepancies observed in the literature regarding the predictive value of capsaicin for differentiating between various causes of cough [21,31,32]. Another problem arises from sample sizes, which are mostly insufficiently powered to significantly distinguish between different disorders. Nevertheless, cough reflex sensitivity to inhaled capsaicin is dose-dependent and highly reproducible [15,21,33] when performed by an individual investigator or laboratory using appropriate methodology [23,33,34]. Therefore, it is useful in the same participant over different periods or after an intervention and has established itself as an important tool in pharmacological and epidemiological studies comparing different populations [15].

Large studies comparing values obtained for capsaicin challenge in healthy controls matched with patients and confirmed by objective testing are still lacking. Several factors must be taken into account. First, females exhibit a heightened cough reflex [35,36], with C2 and C5 consistently lower than in males; the cause of this sex-related difference is unknown, but it also occurs in patients receiving angiotensin-converting enzyme inhibitors [37]. Second, individuals with high cough thresholds who do not reach C5, even when the 500-μM solution is used, should probably be excluded from comparative clinical trials, because a true C5 cannot be discerned [15]; therefore, in population studies, the use of C2 is recommended [15]. Third, although capsaicin challenge provides a model of natural cough in healthy individuals, smoker's cough may confound findings, since it involves airway inflammation [15]. Cigarette smoking exerts a profound effect on the cough reflex and reduces cough reflex sensitivity [38].

In addition, false-negative results may be observed in patients with diabetic neuropathy or familial glossopharyngeal neuropathy [39].

Feasibility and Safety of Inhaled Capsaicin Challenge in Patients With Asthma

A PubMed search using the key words *inhaled capsaicin* AND *asthma* for articles in English with an available full text retrieved 97 results, of which 11 were reviews. Although several of the studies are experimental and involve animals, they do report unprecedented advances that have revolutionized this field.

Animal studies made it possible to firmly establish the role of TRPV1 in airway acid sensing and acid-induced cough [40-43]. This finding is relevant, as tissue acidification has been observed in the exhaled breath condensate of patients with a variety of conditions associated with airway hyperresponsiveness and cough, including asthma and rhinitis [31]. Besides the acid stimulus, TRPV1 channels are sensitized following activation of neuronal receptors for cysteinyl leukotrienes, prostaglandins, histamine, purines, proteases, chemokines, peptides such as nerve growth factor and bradykinin, and many other proinflammatory mediators known to participate in airway inflammatory diseases such as asthma.

Inflammation of the airway is almost always accompanied by a lower threshold to cough response [12]. Protease-activated receptor (PAR) 2 activation, which sensitizes TRPV1, is induced during inflammation [12]. PAR2 agonists do not induce cough per se but potentiate cough induced by TRPV1 activation. Experiments with guinea pigs enable us to speculate that PAR2 causes sensitization to TRPV1 and exaggerates cough in inflammatory airway diseases [44], thus explaining the fact that the capsaicin-induced cough threshold is decreased in patients with asthma [20,45-47]. In addition, proteases such as trypsin and elastase released from leukocytes during inflammation can cleave PARs on afferent neurons, thus activating the receptors and causing neurogenic inflammation [8]. Therefore, it is conceivable that, under inflammatory conditions, TRPV1 undergoes plasticity and upregulation of its expression and function [48].

TRPV1 function in vagal pulmonary myelinated afferents under conditions of chronic airway inflammation induced by allergens has been described in animal models [49-52]. In sensitized guinea pigs, ovalbumin-induced cough responses were inhibited by a TRPV1 antagonist [50]. Moreover, Liu et al [51] demonstrated that the number of coughs induced by inhaled capsaicin increased considerably after antigen challenge in actively sensitized guinea pigs and that the eosinophil count in bronchoalveolar lavage was significantly correlated with the number of coughs caused by capsaicin; on the other hand, it was observed that the antigen-induced increase in cough response is not mediated by bronchoconstrictor response or bronchial hyperresponsiveness. These results were recently confirmed in humans by Ekstrand et al [53], who found that the result of capsaicin challenge in corticosteroid-naïve asthmatic patients correlated significantly with levels of exhaled nitric oxide, but not with methacholine sensitivity. In addition, in OVA-sensitized rats, chronic inflammation was shown to lead to changes in transcriptional patterns, inducing expression of TRPV1 in myelinated airway fibers [52]. Recruitment of additional fibers may lead to long-term changes in neuronal excitability reminiscent of chronic inflammatory states, causing chronic increased chemical and mechanical sensitivity and neuronal excitation even in the absence of chemical stimuli [7]. Neuronal remodeling should therefore be considered a potential additional disease mechanism, occurring in parallel with inflammatory airway tissue remodeling in asthma. In fact, inactivation of capsaicin-sensitive nerves in guinea pigs with chronic allergic pulmonary inflammation was recently shown to reduce pulmonary remodeling [54]. These findings point to the potential role of drugs that block TRPV1 channels, such as the TRPV1 antagonists capsazepine and ruthenium red, which

are of interest in asthmatic patients, who could benefit not only from the short-term action of these drugs in controlling cough symptoms, but also from long-term advantages in reducing remodeling.

Taken together, the results of these experiments lead us to anticipate that capsaicin challenge can confirm the efficacy of corticosteroid-based anti-inflammatory therapy in asthmatic patients. However, this hypothesis has only been examined once to date [55]. Capsaicin challenge was also used to assess the effect of the active type 2 helper T-cell cytokine inhibitor suplatast tosilate on cough response in patients with stable atopic asthma [56] and to assess the benefits of carbocysteine on cough in asthmatic patients [57]. It could also be used to verify the clinical significance of aeroallergen sensitization, as suggested by Weinfeld et al [58], who found that capsaicin cough sensitivity in asthmatic patients sensitized to birch increased during the pollen season. Given its high feasibility as a safe, easy, and inexpensive technique, capsaicin challenge could be considered an option; however, more robust results from larger studies are necessary. Furthermore, it should be considered an important tool for evaluation of patients with unclear respiratory symptoms who do not fulfill the criteria for a diagnosis of asthma [59]. This type of challenge could also be considered a complementary method when investigating different mechanisms of asthma, as its outcome is associated with inflammation markers (and inflammatory mediators can lower the concentration of capsaicin needed for TRPV1 activation [60]), although not with methacholine responsiveness. In bronchial biopsies from patients whose asthma was refractory to therapy with corticosteroids, TRPV1 expression was found to be increased [61], suggesting that, in some patients, an alternative etiopathogenic pathway must be considered. Envirommental and occupational exposure is another important focus of published studies on the applicability of inhaled capsaicin challenge in asthma. Chemicals encountered in the airways are detected by C fibers, which are unmyelinated and therefore allow rapid access for chemical stimuli along the fiber path through the tissue. Since most C fibers are sensitive to capsaicin [7], inhalation challenge has been widely used to study chemical/sensory airway reactivity [62-65].

Patients affected by cough-variant asthma also often show increased sensitivity to capsaicin challenge [65,66], although they present mildly hyperreactive airways and good response to bronchodilators, even if no direct correlation is observed between capsaicin challenge results and airway hyperresponsiveness [25]. However, the mechanisms underlying the symptomatic prevalence of cough in these patients are not well established, and the lack of association between cough sensitivity and bronchoconstrictor responsiveness suggests that it may not be related to the mechanisms modulating airway muscular tone [68]. Antileukotrienes are effective in cough-variant asthma [69,70], although not to the same extent in classic asthma-related cough [71], thus supporting the possibility that cough and bronchoconstriction are modulated by different neural pathways. Nearly 30% of patients develop wheezing and dyspnea in the 3 to 5 years after onset of cough-variant asthma, thus fulfilling the criteria for typical or classic asthma. The remainder probably represent a different phenotype in which leukotrienes are closely involved in the pathogenesis of their chronic cough. As mentioned above, TRPV1, the major receptor of capsaicin, is also activated by cysteinyl leukotrienes. The bronchial mucosa biopsy specimens of patients with cough-variant asthma for whom treatment with antileukotrienes was effective showed a significantly higher proportion of CD63-positive cells in tryptase-positive mast cells [72], which are known releasers of cysteinyl leukotrienes. Therefore, capsaicin challenge could be an appropriate tool for identifying patients who would benefit from this therapy.

Another important role for capsaicin challenge would be to distinguish between patients who could benefit from therapy with inhaled anticholinergic agents. Tiotropium inhibits cough reflex sensitivity to capsaicin, although it has only been studied in patients with acute viral upper respiratory tract infections [73]. Wide variability in the response to ipratropium bromide has been observed in patients with exercise-induced bronchoconstriction [74,75], which is thought to be related to different interpersonal levels of vagal activity [76]. Therefore, it seems reasonable to expect that capsaicin challenge could also be of interest in that field. In fact, with respect to exercise, a very recent study including elite winter sports athletes reported a lower threshold for cough, as assessed by capsaicin challenge: mean (SD) C5 level for athletes was 91 (4) μM in summer, 68 (4) μM in fall, and 42 (5) μM in winter; for nonathletes the values were 123 (5) μM in summer, 145 (4) μM in fall, and 138 (5) μM in winter [77]. In this specific population, parasympathetic dysautonomia was proposed as an etiopathogenic mechanism of asthma [78]. Furthermore, no correlations were found between airway responsiveness to methacholine and cough reflex sensitivity to capsaicin in athletes or in nonathletes. Pretreatment with ipratropium was shown to completely prevent the bronchoconstriction induced by hot humid air in asthmatics [79]. As hyperthermia exerts a direct stimulatory effect on pulmonary sensory neurons, mediated primarily through activation of the TRPV1 channel, capsaicin challenge could play a role in identifying patients who would benefit from this therapy.

Feasibility and Safety of Inhaled Capsaicin Challenge in Asthma-Related Conditions

Besides asthma, the major etiologies of chronic cough are generally accepted to consist of upper airway cough syndrome and GERD [80]. Since both are frequent comorbid conditions of asthma [81], we review the usefulness of inhaled capsaicin challenge in these situations. However, only a small percentage of patients with these very common conditions have chronic cough. Cough hypersensitivity syndrome has been proposed to explain the occurrence of chronic cough in a subgroup of patients exposed to the same putative triggers as the vast majority of the population, whom chronic cough does not affect [82].

Cough Hypersensitivity Syndrome and Airway Sensory Hypersensitivity Syndrome

A subgroup of patients experience asthma-like symptoms after exposure to chemical irritants in daily life, a condition known as airway sensory hyperreactivity [83]. Although the clinical features resemble those of asthma and allergy, objective findings (ie, the results of lung function and bronchial methacholine tests) are normal, and skin prick tests show no sensitization to common aeroallergens. Cough hypersensitivity is a recent concept that helps to explain many of the respiratory symptoms described by patients, in addition to those caused by typical respiratory conditions. No agreed definition of cough hypersensitivity syndrome has been established [82]; it is not clear whether the term airway sensory hyperreactivity syndrome coined by Milqvist et al [83] would include those patients described by Chung et al [84] as having chronic cough hypersensitivity syndrome or those described as having cough hypersensitivity syndrome [85]. In an attempt to clarify this issue, Birring [86] suggested the term cough reflex hypersensitivity to describe what seems to be the same clinical problem. Cough hypersensitivity has been demonstrated in numerous conditions, and it has been proposed that in other respiratory diseases, such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis, many symptoms result from frequently unrecognized cough hypersensitivity [82]. A search of PubMed using the key words inhaled capsaicin and cough hypersensitivity syndrome OR airway sensory hypersensitivity syndrome resulted in 8 full texts.

Studies indicate that symptoms in this subgroup of patients are induced by the sensory nervous system. In fact, patients with asthma-like symptoms who do not present bronchial obstruction cough more after inhalation of capsaicin than do those with asthma and healthy controls [83]. Studies have shown that capsaicin challenge has the ability to mimic symptoms in these patients and that capsaicin challenge is an objective method of demonstrating airway sensitivity to chemical irritants [47,65,87].

Airway sensory hyperreactivity partially resembles multiple chemical sensitivity, a condition that has been classified mainly as a psychological disorder in which symptoms from various organs are induced by low concentrations of diverse chemical inhalants [88,89]. Patients with sensory hyperreactivity have poor quality of life, which is significantly correlated with sensitivity to capsaicin [65]. Confirmation of diagnosis obviates the need for meaningless tests and medication [47]; consequently, any objective method that enables such an approach is of great interest. However, except for Millqvist et al [83], the possibility offered by capsaicin challenge has not been widely explored.

Rhinitis and Upper Airway Cough Syndrome

A search of PubMed using the key words inhaled capsaicin AND rhinitis OR postnasal drip OR upper airway cough syndrome OR sinusitis retrieved only 14 full texts, of which one was a review article and another was a case-report of occupational rhinitis induced by capsaicin.

Two capsaicin-based procedures that have been used in patients with rhinitis are nasal capsaicin challenge and inhaled capsaicin challenge.

Sensory neurons mediate some of the signs and symptoms typically encountered during allergic rhinitis, including itching, sneezing, and reflex-mediated secretion [90], and available evidence shows that patients with allergic rhinitis outside the pollen season more frequently present rhinorrhea in response to nasal challenge with capsaicin than asymptomatic individuals [91]. This likely reflects nasal hyperresponsiveness associated with allergic rhinitis, which involves mucosal end organs including glands, nerves, and blood vessels [92]. In fact, the presence of TRPV1 in human nasal mucosa was observed in peptidergic nerve fibers, epithelial cells, vascular endothelium, and submucosal glands [92,93]. Sensory-autonomic parasympathetic efferent reflex pathways induce secretions from nasal, lacrimal, and salivary glands and dilation of vessels in the nasal mucosa and sinuses [7]. Neuropeptides, such as substance P and CGRP, which are released from chemically stimulated nerve endings, promote neurogenic inflammatory vasodilation and leakage, thus contributing to narrowing or obstruction of the nasal passages [7] and lacrimation, as recently shown in vivo by Alenmyr et al [92]. Activation of nociceptive nerve fibres with hypertonic saline in a human nasal provocation model was reported to induce mucin secretion via release of substance P, suggesting that activation of glandular NK1 receptors may be of importance [94]. The recent findings of TRPV1-positive peptidergic nerve fibers close to MUC5B-positive submucosal glands provides an anatomical substrate for such a neuroglandular interaction in the human nasal mucosa [92].

The sensitivity of the cough reflex when assessed using inhaled capsaicin in patients with seasonal allergic rhinitis increases significantly, not only in untreated patients outside the pollen season compared with healthy volunteers, but also during the pollen season compared to out of season. This is true even if patients are not primarily complaining of cough and regardless of whether they were treated with oral antihistamines [95,96]. Since antihistamines act by blocking the H1 receptor and related symptoms, mucosal inflammation is still present; therefore, it is quite conceivable that they do not change the cough threshold on capsaicin challenge, as previously demonstrated with fexofenadine [97].

These findings highlight a series of issues. First, the observation that patients with seasonal forms of allergic rhinitis still present a highly sensitive cough reflex outside the pollen season is consistent with the previous hypothesis of minimal persistent inflammation that primes the nasal mucosa, leading to increased sensitivity to allergens and nonspecific irritants. In fact, chronic upregulation of inflammatory cells and mediators has been observed in the nasal passage of allergic rhinitis patients during symptom-free periods [98]. In this context, capsaicin challenge would be an easy, safe, and inexpensive method of assessing the efficacy and impact of nasal corticosteroids or antileukotrienes on subclinical inflammation in patients with allergic rhinitis. It would be important for treatment strategies that reduce inflammation during asymptomatic periods to have positive effects on the onset, progression, and severity of the disease [99,100]. To use both nasal and inhaled approaches could be interesting. After capsaicin inhalation challenge targeting the lower airways, the symptom scores for rhinitis increase; a strong correlation

is found between the inhalation response and increased nerve growth factor in nasal lavage fluid [64]. This observation is consistent with the concept of "one airway, one disease" highlighted in the Allergic Rhinitis and its Impact on Asthma guidelines [98].

Another issue that arises is the lower cough threshold in rhinitis patients, which we could speculate to be related to the presence or absence of allergic inflammation. Contrary to expectations based on studies including patients with rhinitis, the results of Nieto et al [21] showed that patients with postnasal drip syndrome had normal sensitivity of the cough reflex. It is therefore likely that allergic inflammation in the lower and/or upper airways triggers neurogenic mechanisms of significant clinical importance, rather than the irritant effect of dripping. However, larger studies should be carried out to validate this hypothesis. Niimi et al [31] actually found that the cough threshold in patients with rhinitis/postnasal drip was much lower than for controls, although their sample size was small [31].

Gastroesophageal Reflux Disease

A search of PubMed using the key words *inhaled capsaicin* AND *gastroesophageal reflux* resulted in 12 full texts, 2 of which are reviews. Most present evidence of how acid can provoke cough in both adults and children [101-103]. Electrophysiological studies on vagal sensory nerves detected acid-induced sustained inward currents with TRPV1-like characteristics, as well as transient acid-sensitive currents, which were probably mediated by sodium-selective acid-sensitive ion channels [7]. However, the relationship between esophageal acidity and asthma has been assessed [21,31,104]. Wu et al [104] showed that cough responsiveness is significantly increased after acid perfusion in the distal esophagus in patients with mild persistent bronchial asthma, even in those without gastroesophageal reflux symptoms or esophagitis; the increased cough sensitivity itself did not cause an asthma attack, and no significant changes were observed in lung function during the periods of acid perfusion. Physicians could be tempted to increase asthma medication in asthmatic patients who complain of cough, without considering the possibility of acid trigger if no GERD symptoms are present. Capsaicin challenge could thus be considered a suitable method for distinguishing patients for whom increasing asthma medication is useless and would expose patients to an unnecessary increased risk of side effects. However, it is noteworthy that, to date, capsaicin challenge has been unable to confirm the diagnosis of asthma or GERD in patients with chronic cough.

Other Conditions

Airway infections are also known to heighten capsaicin cough sensitivity [105,106]. Dicpinigaitis et al [107] showed that viral respiratory tract infection was associated with hypersensitivity to capsaicin inhalation, with a return to normal levels of sensitivity following recovery from the illness. Airway virus-induced inflammation leading to peripheral hypersensitivity of cough receptors induced by mild nonspecific noxious exposure, for example, to atmospheric change has been posited as a reasonable explanation [15,82].

Patients with eosinophilic bronchitis also show increased capsaicin sensitivity, which becomes less pronounced with inhaled corticosteroid therapy [108-110]. Induced sputum examination has been used to diagnose eosinophilic bronchitis because it directly reflects airway inflammation [111]. However, it is relatively labor intensive and requires laboratory support; in addition, its differential power declines in cases of mild inflammation. Although standardized measurement of exhaled nitric oxide has been proposed as useful for ruling out eosinophilic bronchitis in patients with chronic cough [112], it is not available in all centers; therefore, finding a simpler suitable alternative test, such as capsaicin challenge, would be of great value. Further studies are needed to clarify this potential usefulness as a differential test.

It has been proposed that cough sensitivity is affected by allergic processes localized beyond the lower airways [95], since it is more common in patients with atopic dermatitis who do not complain of cough or other respiratory symptoms. Therefore, atopic dermatitis is thought to be accompanied by subclinical inflammation of the airways [113]. Further studies on this subject are needed.

Final Comments

This review provides the latest available information on the use of inhaled capsaicin challenge in asthma and cough-inducing comorbid related conditions. Most studies performed to date have provided interesting data from both animal and human models, and their results for capsaicin challenge are consistent with what is known about the etiopathogenesis of cough-related diseases.

In recent years, it has become clear that parasympathetic sensory neurons innervating the airways contribute to the pathogenesis of asthma. A high parasympathetic tone leading to neurogenic inflammation in the lung is associated with the release and action of neuropeptides leading to an increase in vascular permeability, extravasation of plasma and leukocytes, mucus hypersecretion, airway constriction, and release of further inflammatory mediators. This inflammation constitutes a response of sensory neurons to inflammatory mediators and noxious stimuli and is thought to play a major role in the pathogenesis of asthma. In this review, we present current ideas on the role of TRPV1 channels, the major receptors for capsaicin and the hub of almost all neuronal inflammatory signaling pathways in asthma and related comorbid conditions. Indeed, in several cases, there are strong arguments for considering these channels potential drug targets in asthma. Capsaicin challenge not only contributed to these achievements in the past, but could also have a very important role in future research.

Studies focusing on the critical role of TRPV1 as the irritant sensor in the lung and as a critical determinant of neurogenic inflammation are highly relevant for the asthma patient. Increased knowledge of the role of TRPV1 channels in cells infiltrating the airways of asthma patients will provide us with novel strategies to tackle this disease [7]. The cloning of TRPV1 sparked intensive drug discovery efforts, resulting in the development of novel selective TRPV1 antagonists with

analgesic and anti-inflammatory properties in animal pain models [7,114]. However, the number of studies on the effects of these antagonists in the respiratory system remains limited.

Asthma affects millions of people around the world, and, since the advent of inhaled corticosteroids, few new therapies have been developed to treat such a prevalent disease. Therefore, potential new drugs are regarded with much interest. The latest insights into new pathways lead us to expect that anticholinergics and tachykinin antagonists could be valuable for patients with asthma, although there remains much to learn about the role of capsaicin in identifying patients who could benefit from it. More research into these therapeutic targets is needed.

From a practical point of view, inhaled capsaicin challenge has been neglected. Capsaicin challenge has established itself as a useful objective method for evaluating airway hypersensitivity; however, it is potentially valuable in many other situations, for example, as a diagnostic tool for verifying the clinical significance of aeroallergen sensitization, for evaluation of patients with indeterminate respiratory symptoms, and as a complementary method when investigating different mechanisms of asthma. Capsaicin challenge could also help to determine whether allergic inflammation in the lower and/or upper airways, rather than the irritant effect of drip, is responsible for cough in patients with rhinitis. It could also enable us to rule out eosinophilic bronchitis in patients with chronic cough. This approach could help us to determine the efficacy and follow-up of corticosteroid-based anti-inflammatory therapy in asthma patients by identifying in advance patients with cough-variant asthma who would benefit from antileukotrienes, by distinguishing between asthma patients who could benefit from therapy with inhaled anticholinergic agents, and by assessing the efficacy and impact of nasal corticosteroids and antileukotrienes on subclinical inflammation in patients with allergic rhinitis.

Our study shows that for capsaicin challenge to become a useful procedure in clinical practice in patients with asthma and comorbid conditions, much work has yet to be done. While interesting, publications in this field are scarce; therefore, research should be intensified in the near future.

Although inhaled capsaicin challenge is a very safe noninvasive method for studying cough and sensory airway reactivity, more research is necessary on its applicability in order to ensure greater confidence in its use. Large prospective studies will confirm its suitability for the diagnosis and monitoring of respiratory diseases.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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