

Stress and Airway Reactivity in a Murine Model of Allergic Airway Inflammation

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Key Words

Airway hyperresponsiveness • Airway inflammation • Airway remodeling • Model of stress and asthma • Nervous system of the airways • Stress

Abstract

Airway reactivity is a phenomenon with vast clinical implications in children. The regulation of airway reactivity is influenced by local and central mechanisms. In airway diseases like bronchial asthma, the pathological regulation of the airway caliber causes symptoms like cough and dyspnea. Stress has long been considered a powerful manipulator in the physiological regulation of the airways. To explore potential mechanisms linking stress to the exacerbation of asthma, we developed an animal model that combines allergic airway inflammation and exposure to stress. This review summarizes the experimental data obtained in our and similar mouse models. First, we describe the innervation and neuromediators in the airways, next we analyze the occurrence of airway hyperresponsiveness, and then we explore the phenomenon of stress to finally connect all of the topics in a synopsis.

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Introduction

Wheezing, the clinical correlate for a restricted airway flow, is a common symptom in children [1]. Airway resistance, which determines the airflow, exponentially increases as the radius of an airway decreases [2]. Therefore, the impact of bronchial obstruction is particularly evident in small-diameter airways, e.g. in children. Regulation of bronchial activity can be influenced by many factors. The local factors that adjust the caliber of the bronchi include airway inflammation, whereas the systemic factors include CNS-mediated circadian rhythms and emotional stress. Despite the long-standing clinical assumption about the association of stress with asthma, there was a lack of convincing experimental evidence about mechanisms behind this association. To explore these mechanisms, we developed an animal model by combining allergic airway inflammation with exposure to stress. This review summarizes data determined using our experimental model and models similar to it. To fully explain the findings, we first describe the nervous system of the airway and the mediators involved, next we explore the occurrence of airway hyperrespon-

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siveness (AHR), look at the phenomenon of stress and finally we propose a model connecting all factors discussed.

Nervous System of the Airways

Airway smooth muscles are innervated by sympathetic and parasympathetic nerves [3]. Activation of parasympathetic cholinergic nerves induces contraction, whereas sympathetic activation leads to relaxation of the smooth muscles. Noradrenergic sympathetic nerves are found in the airways of all species [3, 4]. There is little evidence demonstrating functional sympathetic innervation of the human airway smooth muscles; the main pathway for sympathetic effects is through the action of circulating adrenaline on β_2 -receptors expressed on airway smooth muscles [5]. In addition to the cholinergic and adrenergic systems, other pathways are important for the functioning of airways [6]. Afferent nerve fibers and efferent non-cholinergic parasympathetic nerves trigger airway smooth muscle constriction and relaxation, respectively. In the airways, afferent nerve fibers lining the mucosal epithelium secrete tachykinins such as substance P (SP) and neurokinin A (NKA) [7, 8]. SP-positive nerve fibers innervating the lower airways are mainly derived from cell bodies located in the jugular-nodose ganglia [9]. Both SP and NKA were shown to enhance bronchoconstriction in asthmatics [10–12], but only SP, and not NKA, induced airway hyperreactivity to methacholine in asthma. The effects of tachykinins on the airways are mediated by neurokinin (NK) receptors. Two of the three tachykinin receptors, namely the NK-1 and NK-2 receptors, have been detected in human bronchi and subpleural lung [13]. In situ hybridization demonstrated the expression of NK-1 receptor mRNA in submucosal glands and airway epithelial cells [14]. Contraction of airway smooth muscles in the guinea pig is mediated mainly by NK-2 receptors [15, 16], whereas the NK-1 receptor mediates proinflammatory and vascular effects [17–19]. Enhanced expression of the NK-1 receptor [14] as well as of the NK-2 receptor [13] has been observed in lung tissue taken from asthmatic subjects. In another study, asthmatic subjects were shown to express increased amounts of epithelial SP and NK-1 receptor, the former correlating well with enhanced mucus production in the airway epithelium [20]. However, although SP and NKA are released in asthmatic airways and induce specific effects, their role in the inflammatory process of asthma is far from clear.

Airway Hyperresponsiveness

In addition to bacterial and viral infections, allergic asthma is a common cause of airflow limitation within preschool age children. Asthma is a chronic disease characterized by airway inflammation, increased AHR and episodes of airway obstruction [21]. AHR has been defined as increased bronchial reactivity to common stimuli such as cold air, hypertonic saline, or irritants. Clinical experience in evaluating the AHR identified two different manifestations of AHR: one transient and one persistent [22, 23]. While the transient AHR is strongly associated with the airway inflammation, the persisting AHR is believed to be dependent on airway remodeling. Both types of AHR can be discerned by the level of reaction to different stimuli such as metacholine or histamine.

Airway Inflammation

Since the first observation of a seasonal increase in AHR in subjects with pollen-induced allergic asthma, a consistent body of data pointing to a causal relationship between inflammation and AHR has accumulated [24]. The evidence includes animal studies demonstrating the requirement for immune cells in onset of allergic asthma [25, 26]. Similarly, human studies demonstrated association between the number of immune cells and the level of AHR [27], whereas intervention studies showed that a reduction of inflammation reduces AHR [28]. The concept of inflammation-induced AHR has been extended to airway inflammation caused by viral and bacterial agents and to occupational circumstances. Allergic airway inflammation is characterized by an influx of lymphocytes, monocytes and eosinophils into the lung and presence of a T-helper cell type 2 (Th2)-type immune response and elevated IgE levels in serum. Besides Th2 cytokines that orchestrate cell recruitment and inflammatory changes, other mediators are released during inflammation. Because of their capacity to induce neurogenic inflammation, bronchoconstriction, and vascular leakage tachykinins have been proposed to play an important role in asthma [29, 30]. In allergic airway inflammation, tachykinins are released into the airways after exposure to allergen. Higher levels of SP have been found in bronchoalveolar lavage (BAL) fluid of atopic subjects compared to non-atopic individuals. These differences were further increased by allergen provocation [31]. In a guinea pig model of allergic airway inflammation, a sig-

nificant increase in SP was detected in lung tissues after allergen challenge [32]. SP found in the airways presumably derives from afferent nerve fibers lining the mucosal tissue. Additionally, inflammatory and immune cells that are attracted to the airways during allergic inflammation, e.g. eosinophils [33–35], macrophages [36], lymphocytes [37] and dendritic cells [38], are able to secrete tachykinins in response to various stimuli. SP modulates a variety of immune responses including mast cell degranulation [39], chemotaxis of eosinophils, neutrophils [40–42] and lymphocytes [43] and interleukin (IL)-2 production by human and murine T cells [44, 45]. Despite these compelling data, in contrast to cytokine-related data, very little is known about the role of neuropeptides in allergic asthma, thus, further studies are indicated.

Airway Remodeling

Airway remodeling is characterized by non-reversible structural changes that develop secondary to chronic airway inflammation. Histologically, airway remodeling leads to a subendothelial fibrosis, changes in extracellular matrix composition and hypertrophy and/or hyperplasia of airway smooth muscle resulting in an enhanced airway wall thickness. Also, there is data for a functional change in the nerve system of the lung after inflammatory processes. Research of the recent years has developed a concept of 'peripheral neuronal plasticity'. It has been shown that neurons of the autonomic nervous system change their transcriptome composition and start to produce proinflammatory mediators such as SP and NKA. In a guinea pig model of allergic airway inflammation, sensitization and provocation resulted in *de novo* expression of pre-pro-tachykinin mRNA (SP) in the ganglia of sensory neurons [46, 47].

Stress

Stress can be defined as a stimulus that activates both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. This activation induces humoral and neuronal changes that may finally lead to an adaptation of the individual to the changed environment. Recent animal studies indicate that stress may further lead to central and peripheral release of neuropeptides, especially the tachykinin SP [for review, see 48], and thereby induces or exacerbates inflammatory processes [49–52].

Stressed mice showed elevated immunoreactive SP in peritoneal wash fluid and increased IL-6 secretion from peritoneal macrophages. The stress-induced augmentation of IL-6 secretion was prevented by depletion of sensory neurons with capsaicin or by application of a NK-1 receptor antagonist [53]. In rats, neonatal denervation of afferent SP-containing nerve fibers with capsaicin reduced stress-provoked activation of mast cells, which appear to play a significant role in the pathophysiology of stress-sensitive skin disorders and interstitial cystitis [54, 55]. SP mediation in stress-promoted inflammation has also been demonstrated in animal models of abortion and hair growth inhibition [56–58].

There are broad clinical and experimental data showing that stress can worsen asthma. Patients exposed to acute stressors show altered lung function [59, 60]. Furthermore, an increased airway inflammation was shown by Liu et al. [61], who found that final examinations led to increased eosinophilic airway inflammation in students with asthma. However, the mechanisms linking stress to asthma are not well understood.

Model of Stress and Asthma

To explore mechanisms linking stress with chronic constrictive airway diseases, in recent years murine models with use of emotional stress and allergic airway inflammation were developed by some researchers. Airway reactivity (AR) of mice can be assessed with different *in vivo* and *ex vivo* methods. The *ex vivo* technique of electrical field stimulation (EFS) of tracheal smooth muscle segments is best suited to dissect neuronal dysregulation of the airways [62]. Forsythe et al. [63] used a model where experimentally-induced allergic airway inflammation was combined with acute restraint stress for 3 consecutive days during the time of immunological challenge. To induce chronic stress, restraint and forced swim stress were combined and repeated for several days. AR to aerosolized methacholine was measured *in vivo* using the Buxco whole-body plethysmograph challenging mice with increasing concentrations of metacholine. The enhanced pause was calculated as an indicator of AR [64]. Although they found enhanced airway inflammation depending on the duration of stress, there was no augmentation of AR after stress exposure. This might be due to the applied stressor or the method the group used to evaluate AR. On the other hand, Chida et al. [65] used foot-shock stress directly or via a communication box to induce psychological stress in animals watching the foot-

shock stress group. In this particular setting, stress was applied several weeks before sensitization and challenge. AR measured with the Buxco whole-body plethysmograph was found to be significantly higher in both stress groups than in the control group. These results were confirmed by AR measurement with EFS. To prove the involvement of the HPA axis and corticosterone, Chida et al. [65] applied the glucocorticoid receptor antagonist RU-468 1 h prior to antigen challenge. RU-468 pretreatment reduced the effect of psychological stress on AR but not the effect of foot shock. The above findings together with the fact that corticosterone concentration drops in response to the allergen challenge in mice subjected to psychological stress suggest an association of HPA axis with early-life stress and murine adult asthma.

We developed an animal model that combines allergic airway inflammation and exposure to sound stress [66]. In this model, mice are sensitized to chicken ovalbumin (OVA), which induces synthesis of OVA-specific IgE. To induce an allergic airway inflammation, mice are challenged twice with OVA aerosol. At the time of first aerosol challenge, mice are exposed to sonic stress for a 24-hour period. Sonic stress is emitted by a rodent-repellent device at a frequency of 300 Hz (sound pressure level 75–80 dB) for 1 s each time, in irregular intervals of about 15 s. The stress device is placed in the home cage so that the mice are not able to escape perception of the sound. Meanwhile, control mice remain in a room undisturbed by sound. Sonic stress has been used in various animal models as an effective inductor of stress reactions [67–69]. It is based on pilot experiments employing stress challenge ranging from 45 min, 2, 4, 12, 24, and 48 h of sound, which led to appreciate 24 h of sound challenge as a moderate stressor. Other stressors, i.e. restraint, have a more profound effect, i.e. on the skin immune system, where 2 h suffice to mirror the results seen after 24 h of sonic stress [70]. The method to evaluate AR, EFS, utilizes electrical impulses to induce muscle constriction via neuronal structures of the tracheal smooth muscle segments, namely cholinergic and sensory neurons [62]. Because of the anatomical seizure of the tracheal segment, higher neuronal structures can be excluded as causing the bronchomotoric action.

Twenty-four hours after the second allergen challenge, both stressed and non-stressed sensitized mice show AR. However, there is a significant difference between the groups demonstrating that airway constriction in stressed mice can be stimulated at lower frequencies, which have no effect on airways of non-stressed mice [66]. There is data suggesting that airway hyperreactivity is mediated

via neuronal pathways and might even exist independently of airway inflammation. Thus, in animals that lack sensory neurons due to capsaicin pretreatment, AHR does not develop [71, 72]. In addition, AHR can be detected in asthmatic individuals without histological signs of airway inflammation [73] or in individuals with long-term suppression of inflammation [74]. Thus, stress might exert a direct influence on neuronal structures resulting in enhanced AR.

To dissect the possible role of SP in allergic asthma, we applied an NK-1 receptor antagonist (RP-67580) before and after stress exposure. AR was assessed by EFS. In stressed animals, the application of RP-67580 resulted in a significant decrease of AR by 35% [75]. Currently, only limited clinical data are available on the effect of tachykinin NK-1 receptor antagonists on allergic bronchial asthma. Studies have focused on the antagonist's effect on bronchoconstriction, which has been rather poor [76, 77], whereas influence on airway inflammation has not been examined. The results of our animal study suggest that NK-1 receptor antagonists might attenuate the stress-induced component of AR. Further studies will be necessary to provide evidence for a similar regulation in humans, hopefully resulting in new treatment options.

Our work with NK-1 receptor antagonist RP-67580 encouraged us to the search for possible sources of SP in our model. Therefore, using retrograde neuronal tracing in combination with immunohistochemistry we analyzed the expression of SP in airway-specific and overall vagal sensory neurons of the jugular and nodose ganglion complex. Sensory neurons in the jugular-nodose ganglia innervating the airways were retrograde labeled with a fluorescent dye. Stress was applied to non-sensitized and sensitized/challenged animals. Stress and/or allergic airway inflammation significantly increased SP expression in retrograde labeled vagal sensory neurons from the mouse lower airways, as compared to controls. This result suggests that the central event of exogenously applied stress is a stimulus for the induction of SP in sensory neurons. Similarly, SP expression increased in overall vagal sensory neurons identified by the neuronal marker protein gene product (PGP) 9.5. Qualitative immunohistochemical analysis of SP expression in the airways demonstrated predominant presence of SP in the nerve fibers innervating airway vasculature. Further, immune cells surrounding the airways also express SP [78]. Therefore, the increasing effect of stress on airway inflammation might be in part mediated by SP originating from immune cells that infiltrate lung tissue during allergic airway inflammation, synergizing with neuronal SP release.

Using our animal model, we found evidence for an enhanced allergic airway inflammation after application of stress. Associated with this increase, we found that the percentage of TNF- α ⁺ CD3⁺ T cells in lung draining lymph nodes was higher in stressed mice and mice after application of SP. In contrast, the influence percentages of IFN- γ ⁺ CD3⁺ cells, whereas the application of SP increased the percentages of IFN- γ ⁺ CD3⁺ cells [79].

SP-immunoreactive nerve fibers in tracheobronchial lymph nodes have been reported [80]. Moreover, SP might be released by afferent sensory neurons after stress, as has been hypothesized to occur in stress-related dermatoses [81] and hair loss [67]. SP-mediated TNF- α production has been noted in peritoneal macrophages in response to cold water stress [53], and in a murine model of stress-induced abortion [57, 82]. Thus, the higher percentage of TNF- α ⁺ T cells in local lymph nodes observed in stressed animals with increased airway inflammation could be due to SP release from nerve fibers innervating local lymph nodes. Interestingly, SP application induces a comparable increase in inflammatory cell number in BAL together with a high percentage of TNF- α ⁺ T cells in draining lymph nodes. This suggests a stress-mediating role for the tachykinin SP in our model of stress-exacerbated airway inflammation. Lately, we were able to further evidence supporting the significance of TNF- α in the mediation of stress-related pathology by showing that in human subjects suffering from asthma, the amount of TNF- α ⁺ peripheral blood T cells correlates with the self-reported stress perception [83].

Furthermore, we looked at the cell influx of eosinophils into the airways, which is enhanced in stressed mice. Eosinophil trafficking in allergic airway inflammation is mediated by a complex multistep process, in which locally expressed cytokines, chemokines and adhesion molecules result in the extravasation of inflammatory and immune cells into the airway mucosa and lumen. Therefore, we measured the concentration of the

chemokine eotaxin and of the cytokines IL-4 and IL-5 in BAL fluid and studied expression of adhesion molecules on eosinophils. Stress significantly increased allergen-induced airway inflammation as identified by leukocyte numbers in BAL fluids. Eotaxin levels from stressed mice were significantly higher 24 h after stress [84]. The enhanced expression of eotaxin might be caused by stimulation of airway epithelium or inflammatory cells via neuronal mediators released after stress. Corroborating this, the release of SP from airway sensory neurons stimulates bovine bronchial epithelial cells to express neutrophil chemoattractant mediators, therefore increasing neutrophil adherence [85, 86]. Numao and Agrawal [41] demonstrated that neuropeptides like SP potentiate in vitro the chemotactic effect of platelet-activating factor and leukotriene B₄ on eosinophils isolated from allergic patients, suggesting a priming effect of neuropeptides released from sensory nerves in response to neurogenic stimuli. Alternatively, a direct release of eotaxin from neurons might be responsible for increased concentration of eotaxin in our model, as eotaxin-positive airway neurons have been shown recently in a model of allergic airway inflammation in monkeys [87]. No differences were found for vascular or cellular adhesion molecule expression or cytokine levels.

In conclusion, our model of combined stress and allergic airway inflammation has provided evidence that beside the classical stress mediation via the HPA axis, there are exciting new communication pathways induced by stress and influencing the respiratory system. These pathways utilize neuronal elements and are able to mediate between CNS-conceived stimuli and the immune system resulting in a stark impact on the bronchial caliber. These data not only provide an explanation for the clinically observed stress-induced exacerbation of bronchoconstriction but also open new potential therapeutic avenues for the manipulation of stress-induced neuroimmune cross-talk.

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