Inhaled Ammonium Persulphate Inhibits Non-Adrenergic, Non-Cholinergic Relaxations in the Guinea Pig Isolated Trachea

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Key Words

Ammonium persulphate • Inhibitory non-adrenergic, non-cholinergic innervation • Neurotransmitters • Trachea • Guinea pig • Occupational asthma • Airways

Abstract

Background: Persulphates can act both as irritants and sensitizers in inducing occupational asthma. A dysfunction of nervous control regulating the airway tone has been hypothesized as a mechanism underlying bronchoconstriction in asthma. **Objectives:** It was the aim of this study to investigate whether inhaled ammonium persulphate affects the non-adrenergic, non-cholinergic (NANC) inhibitory innervation, the cholinergic nerve-mediated contraction or the muscular response to the spasmogens, carbachol or histamine, in the guinea pig epithelium-free, isolated trachea. **Methods:** Male guinea pigs inhaled aerosols containing ammonium persulphate (10 mg/m³ for 30 min for 5 days during 3 weeks). Control animals inhaled saline aerosol. NANC relaxations to electrical field stimulation at 3 Hz were evaluated in whole tracheal segments as intraluminal pressure changes. Drugs inactivating peptide transmission, nitric oxide synthase, carbon monoxide production by haem oxygenase-2 and soluble guanylyl cyclase were used to assess the involvement of various inhibitory neurotransmitters. Carbachol and

histamine cumulative concentration-response curves were obtained. **Results:** In both groups, nitric oxide and carbon monoxide participated to the same extent as inhibitory neurotransmitters. In exposed animals, the tracheal NANC relaxations were reduced to $45.9 \pm 12.1\%$ ($p < 0.01$). The cholinergic nerve-mediated contractions to electrical field stimulation and the muscular response to histamine were not modified by ammonium persulphate exposure. The muscular response to carbachol was unaffected up to 1 μM . Conversely, the response to the maximal concentration of carbachol (3 μM) was increased ($p < 0.01$). **Conclusion:** Ammonium persulphate inhalation at high concentrations impairs the nervous NANC inhibitory control in the guinea pig airways. This may represent a novel mechanism contributing to persulphate-induced asthma.

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Introduction

Several etiological agents of occupational asthma, such as isocyanates, glutaraldehyde and persulphates, are irritants at high concentrations, whereas they behave as sensitizing agents in the case of chronic exposure at low concentrations [1]. Persulphate salts are widely used, due to their high reactivity, in several manufacturing pro-

cesses, in the chemical, pharmaceutical, metallurgic, textile, photographic, food and cosmetic industries. Though the epidemiology of persulphate-induced asthma is ill-defined [2, 3], it has been estimated that it may represent up to 4% of all occupational asthma cases [4]. In particular, persulphates are the major agents causing occupational asthma in hairdressers [3, 5].

An impairment of nervous control regulating the airway contractility has been hypothesized as a mechanism underlying the dysregulation of airway smooth muscle tone in asthma [6, 7], but its role is still undefined [8]. A recent study by our group showed that adenosine, which is a mediator of several inflammatory processes, can impair the non-adrenergic, non-cholinergic (NANC) relaxation in the guinea pig isolated trachea by activating two distinct receptors [9]. The contribution of transmission by vasoactive intestinal peptide (VIP), nitric oxide (NO) and other putative neurotransmitters to nerve-mediated relaxant responses of mammalian airway smooth muscle is not yet established, in both normal and pathological conditions [6]. Recently, a study conducted by our group has revealed the participation of carbon monoxide (CO), produced by action of the enzyme haem oxygenase-2 in the NANC relaxation of the guinea pig trachea [10].

Structural and functional changes in nerves have also been suggested as a major focus for the research on the pathogenesis of airway disorders [11], in particular with the use of animal models of irritant-induced asthma [12].

The pathogenesis of persulphate-induced asthma is not clearly elucidated. Humoral immune-mediated mechanisms are thought to play a role, but immunoglobulin E participation is still undefined [2, 5]. Cellular immune-mediated mechanisms, involving mast cell participation have also been hypothesized [13].

Direct irritation and damage of the airway mucosa is considered a main factor in the pathogenesis of non-allergic occupational asthma due to various agents [14]. A dysregulation of the intrinsic innervation of the airways has also been proposed to contribute to different forms of asthma [15–19]. A potential dysregulation of NO-mediated inhibitory responses as a cause of airway dysfunction due to a chronic exposure to oxidizing substances has been hypothesized but not yet demonstrated [20].

In mimicking the physiological abnormalities of human asthmatics, guinea pigs may be seen as an ideal model as they develop well-characterized early- and late-phase reactions to allergen challenge following sensitization. Again, most of the focus on early- and late-phase reactions by the airways in guinea pigs has been related to responses

to irritants rather than to classical 'allergic asthmatic' reactions. An interesting feature of the guinea pig acute response to chemical irritants is the extreme nature of the response [21]. Conventionally, the guinea pig has been the species of choice for the toxicological evaluation of chemical related respiratory allergy, primarily because it is possible to elicit and measure, with relative ease, challenge-induced pulmonary reactions that resemble the acute clinical manifestations of human allergic asthma [22]. Repetitive allergen provocation in mice has been shown to mimic important features of the human disease and would appear to represent an improvement over the acute challenge models [23]. That is why we designed a 3-week exposure protocol to investigate the effects of ammonium persulphate inhalation. Since the persulphate threshold limit value (TLV) proposed by the American Conference of Governmental Industrial Hygienists is 0.1 mg/m^3 , we assumed that humans are not usually exposed to concentrations exceeding this limit. However, a cross-sectional study [24] did not show an increased risk of occupational asthma in a population of workers exposed to persulphate concentrations of about 1 mg/m^3 . Higher concentrations, up to 500 mg/m^3 , were used in animal studies [25]. Accidental exposure to uncontrolled high concentrations of irritant chemicals can lead to irritant-induced asthma, also known as reactive airways dysfunction syndrome [26]. We chose a concentration that was 100 fold the TLV-time weighted average, aiming to elicit both sensitizing and irritant mechanisms.

The aims of the present study were to investigate: (1) whether ammonium persulphate inhalation impairs NANC relaxation in the guinea pig isolated trachea; (2) whether ammonium persulphate inhalation affects the participation of NO, VIP and CO in the NANC tracheal relaxations to electrical field stimulation (EFS), according to the method first described by Tanihata and Uchiyama [27], and (3) whether ammonium persulphate exposure affects the cholinergic nerve-mediated contraction or the muscular response to exogenous carbachol or histamine.

Materials and Methods

Animals

Male albino guinea pigs weighing 500–600 g (Bettinardi, Momo, Italy) were used. Guinea pigs were housed in standard animal facilities, providing constant temperature ($21 \pm 1^\circ\text{C}$), relative humidity (50–55%) and alternating 12-hour light and dark cycles. Animals were provided with food and water ad libitum. Care and handling of the animals were in accordance with the

European Union Directive 86/609 and the National Institutes of Health recommendations for the humane use of animals. All experimental procedures were reviewed and approved by the appropriate Animal Use Committee of the University of Pavia, Italy, where the experiments were performed. The number of animals used was kept to the minimum necessary for a meaningful interpretation of the data and animal discomfort was kept to the minimum. For functional studies, animals were killed by cervical dislocation and rapid exsanguination.

Exposure to Ammonium Persulphate

The animals were exposed to ammonium persulphate by inhaling aerosols of aqueous solution of the substance at irritating concentrations, 10 mg/m³, superior to the TLV of 0.1 mg/m³ (American Conference of Governmental Industrial Hygienists) for 30 min for 5 consecutive days during 3 consecutive weeks. The aerosol was generated by an ultrasonic nebulizer (DeVilbiss Ultra Neb 2000, particle size range 0.5–5 µm, mean 2.8 µm, with an output settable from 0 to 7.5 ml/min solution) connected to a plexiglas exposure chamber (5 litres) with continuous air flow. Control animals inhaled saline aerosol.

Functional Studies

Twelve hours after the last exposure, the trachea was excised and transferred to a Petri dish containing oxygenated (95% O₂ and 5% CO₂) standard Tyrode solution. A 3-cm-long tracheal tube was prepared by gently removing the mucosa [28], to avoid the electrical stimulation of epithelial cells, which may release substantial amounts of prostanoids, ATP and NO, that may distort the nerve-mediated response to EFS [10]. Since this technique allows a histologically proved total mucosa ablation, the use of prostanoid synthesis inhibitors (e.g., indomethacin) is no longer needed. Then, the tracheal tube was cannulated at each extremity using 2 polyvinyl chloride tubes (outer diameter 2.0 mm, inner diameter 1.35 mm) and was set up horizontally in a 10-ml organ bath containing Tyrode solution, maintained at 37°C and bubbled with a mixture of 95% O₂ and 5% CO₂. The preparation was flushed intraluminally with a peristaltic pump delivering Tyrode solution at 0.4 ml min⁻¹ for 30 min. Then, one end of the preparation was occluded and the other one was connected to a pressure transducer for intraluminal pressure recording. Signals were recorded using a PowerLab data acquisition system (ADInstruments Ltd., Crowborough, UK) and analyzed using PowerLab Chart version 4.1.1 software.

After 1 h of equilibration, the tracheal tube was stimulated via 2 platinum electrodes placed in parallel, 1 cm apart, and connected to an electrical stimulator (MARB ST 87). Trains of rectangular pulses (0.5 ms duration, 0.3–30 Hz frequency at 60 V) were delivered for 5 s at 10-min intervals. EFS-induced NANC relaxations were measured as reduction in the intratracheal pressure. They consisted of an initial fast response (peak response) followed by a late slow recovery of the tone up to the basal value (late response). The overall inhibitory response was evaluated as area under the curve (AUC) and calculated as the integral from baseline for each response (Pa · s). All experiments were carried out under NANC conditions, in the presence of hyoscine (1 µM) to block muscarinic acetylcholine receptors, piperoxan (1 µM) and of propranolol (1 µM) to block α- and β-adrenoceptors. NANC relaxations to EFS were elicited before (control) and 45–60 min after the single administration of the following drugs: the

NOS inhibitor, L-NAME (100 µM); the endogenous peptide digesting enzyme, α-chymotrypsin (2 U ml⁻¹) to assess the possible participation of peptide mediators (like VIP) in the response; the haem oxygenase-2 inhibitor, zinc protoporphyrin-IX (ZnPP-IX, 10 µM) to block CO formation; and the soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 µM) to block the cGMP-related intracellular signalling pathway common to NO and CO. Other experiments were conducted by concomitantly giving L-NAME and ZnPP-IX or L-NAME, ZnPP-IX and ODQ to inhibit NO- and CO-producing enzymes and to prevent the formation of cGMP, the second messenger involved in smooth muscle relaxation. Since ZnPP-IX is photosensitive, all experiments using this substance were performed in complete darkness [29]. The neurotoxin tetrodotoxin (TTX, 1 µM) was given in a subset of experiments to ensure that EFS-evoked inhibitory responses were neurogenic.

In a separate subset of experiments, untreated tracheal segments were electrically stimulated to assess cholinergic nerve-mediated contractions measured as intratracheal pressure increase. In the same preparations, cumulative concentration-response curves to spasmogens were constructed by adding to the bath increasing concentrations of carbachol (1 × 10⁻⁸ to 3 × 10⁻⁶ M) or histamine (1 × 10⁻⁸ to 1 × 10⁻⁴ M), both in the control and persulphate-treated tracheal segments. We carried out 6 experiments, both in control and persulphate-exposed animals, by adding 100 µM ammonium persulphate to the organ bath. The tracheal preparations were stimulated as described above, before and after (45 min) ammonium persulphate addition.

Histology

To ensure that the adopted exposure protocol is able to cause inflammation in the airway mucosa, segments of excised tracheae were fixed with 10% formaldehyde, processed with 70–100% ethanol, then with xylene, enclosed in paraffin, cut into 5-µm-thick slices by a microtome and finally stained with haematoxylin-eosin to verify the presence of inflammatory alterations. Total cell count was used for a semi-quantitative evaluation of the inflammatory cell infiltration in the mucosa. Ten fields, each corresponding to a real section area of 0.014 mm², were randomly picked in both the control and persulphate-exposed tracheal sections.

Statistical Analysis

Data were analyzed as raw data (AUC) and expressed as the mean ± SEM of the percent residual response after treatment compared with control response (100%). Statistical analysis was performed by means of Student's *t* test for paired or unpaired data or analysis of variance followed by Bonferroni's test for multiple comparisons. A *p* value <0.05 was considered statistically significant.

Drugs

α-Chymotrypsin, hyoscine, isoprenaline, L-NAME, propranolol, TTX (all purchased from Sigma-Aldrich, St. Louis, Mo., USA) and piperoxan (Rhône-Poulenc, Courbevoie, France) were dissolved in distilled water. ZnPP-IX (Sigma-Aldrich) was prepared (and maintained) in the dark by first dissolving it in 0.2 N sodium hydroxide solution and then diluting in distilled water. The pH of the solution was adjusted to 7.4 with 0.2 N HCl. ODQ (Sigma-Aldrich) was prepared by first dissolving it in dimethylsulfoxide and then diluting in distilled water.

Fig. 1. a Frequency-dependent relaxant responses at 0.3–30 Hz, represented as the mean $\pm$ SEM of the AUC in 10 experiments. **b** Representative tracing showing EFS-induced NANC relaxations evoked by 3 Hz in the guinea pig isolated whole trachea, in the absence and in the presence of TTX.

Results

Under NANC conditions, EFS at 0.3–30 Hz induced relaxant responses, consisting of an initial fast phase (peak response) followed by a late slow response up to the recovery of the basal tone, which were reproducible over a 7-hour time interval and were abolished by 1 μ M TTX ($n = 4$) (fig. 1b). We chose to study the relaxant responses at 3 Hz because they were the closest to 50% of the maximal electrically induced relaxation at 30 Hz (fig. 1a). EFS-induced relaxations were frequency dependent and sub-maximal compared with the response evoked by 10 μ M isoprenaline (peak response 45 and 67%, at 3 and 10 Hz, respectively; $n = 10$).

In isolated tracheal segments from exposed animals, the NANC relaxations were significantly reduced. In particular, the AUC was $45.9 \pm 12.1\%$ ($p < 0.01$), as compared with the control (fig. 2). On the other hand, the baseline pressure in the unstimulated trachea did not differ between the 2 groups. In particular, after occluding one end of the preparation, the recorded maximal pressure was 223.4 ± 79.1 Pa in the control group ($n = 10$) and 150 ± 50.5 Pa in the persulphate-exposed group ($n = 10$). The relaxations induced by isoprenaline in the 2 groups were also similar. In particular, the pressure fall was $1,113 \pm 213$ Pa in the control group and $1,234 \pm 444$ Pa in the exposed group ($n = 10$ in both groups).

The histologic study of the tracheal segments showed the presence of a marked inflammatory infiltration in the mucosa of persulphate-exposed animals (fig. 3). The mean total cell count was 7.2 ± 0.9 per field in the control sections, whereas it was 34.5 ± 3.9 in the persulphate-exposed sections ($p < 0.001$). The cells infiltrating the mucosa of persulphate-exposed animals were mostly

Fig. 2. EFS-induced NANC relaxations evoked by 3 Hz in isolated whole trachea were evaluated as AUC of the overall inhibitory response (including both the fast and the slow component) in both controls and persulphate-exposed animals. Data represent the mean $\pm$ SEM of 10 experiments. * $p < 0.01$.

neutrophils, with an eosinophil proportion of $21.5 \pm 2.4\%$.

In the control group, treatment with α -chymotrypsin (2 U ml^{-1}) did not affect NANC relaxations evoked at 3-Hz stimulation. This is in line with the evidence that peptide transmission does not participate in the NANC response when evoked at low frequency of stimulation [10, 27]. In spite of its low specificity, α -chymotrypsin has been, and still is, widely used in the pharmacological setting to assess the peptidergic nature of some neurotransmitters (VIP and related peptide family) involved in NANC inhibitory transmission. L-NAME (100 μ M) reduced NANC relaxations by about 40% ($p < 0.05$). As a single treatment, ZnPP-IX (10 μ M) failed to affect NANC relaxations. However, when ZnPP-IX was added in the bath concomitantly with L-NAME, a reduction in NANC relaxations significantly greater (about 70%, $p < 0.05$)

Fig. 3. Inflammatory infiltration of the tracheal mucosa in a persulphate-exposed animal (a). The mucosa from a control animal is shown for comparison (b). $\times 20$.

Fig. 4. Effects of various pharmacological treatments with single drugs [α -chymotrypsin (α -Ct), ZnPP-IX, L-NAME, ODQ and TTX] or with a combination of drugs (L-NAME + ZnPP-IX or L-NAME + ZnPP-IX + ODQ) on EFS-induced NANC relaxations evoked by 3 Hz in the guinea pig isolated whole trachea in control and persulphate-exposed animals. α -Chymotrypsin (2 U ml^{-1}) and ZnPP-IX ($10 \text{ }\mu\text{M}$) were ineffective, whereas L-NAME ($100 \text{ }\mu\text{M}$) significantly reduced the AUC of the inhibitory responses. L-NAME + ZnPP-IX significantly reduced the AUC of NANC re-

sponses either versus control relaxations or versus L-NAME alone. The effect of ODQ alone was similar to that of L-NAME + ZnPP-IX. The combination of L-NAME + ZnPP-IX + ODQ inhibited the responses to an extent similar to TTX ($1 \text{ }\mu\text{M}$). In exposed animals, the NANC relaxations were reduced by L-NAME, ZnPP-IX and ODQ, alone and in combination, to an extent similar to controls. Data are expressed as the percent of baseline AUC and represent the mean $\pm$ SEM of 5–8 experiments. ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.05$ versus baseline; ^d $p < 0.01$ versus baseline.

Fig. 5. Cholinergic nerve-mediated contractions in isolated whole trachea were superimposable both in control and persulphate-exposed animals. Contractions were measured as maximum intratracheal pressure increase (Pa). Data represent the mean $\pm$ SEM of 5 experiments.

Fig. 6. Cumulative concentration-response curves to histamine (1×10^{-8} to 1×10^{-4} M) in isolated whole trachea were superimposable both in control and persulphate-treated animals. Contractions were measured as maximum intratracheal pressure increase (Pa). Data represent the mean $\pm$ SEM of 5 experiments.

than that caused by L-NAME alone was observed. The extent of the reduction caused by ZnPP-IX and L-NAME was similar to that produced by $10 \mu\text{M}$ ODQ. An additional series of experiments was carried out to assess the effect caused by the inhibition of NO- and CO-producing enzymes and soluble guanylyl cyclase to prevent the formation of cGMP, the second messenger common to both NO and CO systems. In the presence of L-NAME, ZnPP-IX and ODQ, NANC relaxations were inhibited by about 90%, a value similar to that caused by $1 \mu\text{M}$ TTX (fig. 4). This observation shows that almost the overall NANC relaxation in the guinea pig isolated trachea is due to NO and CO release from intrinsic NANC inhibitory innervation [10].

As mentioned above, in the tracheal preparations from ammonium persulphate-exposed animals, the NANC relaxations were approximately halved compared with control. L-NAME, ZnPP-IX and ODQ, both alone and in combination, percentually reduced the residual relaxations with the same proportion as in controls (fig. 4).

The excitatory responses to nerve stimulation at 1, 3, 5 and 10 Hz were frequency dependent. These responses were abolished by either hyoscine or TTX ($1 \mu\text{M}$ each; $n = 4$) indicating a cholinergic nerve-mediated contraction. Both nerve-mediated and muscular contractions to exogenous histamine (10 nM – $100 \mu\text{M}$) were not modified by the exposure to ammonium persulphate (fig. 5, 6). The muscular response to exogenous carbachol (10 nM – $3 \mu\text{M}$) was unaffected up to $1 \mu\text{M}$. Conversely, the response to the maximal concentration of carbachol ($3 \mu\text{M}$) was increased by 90% ($p < 0.01$) following ammonium persulphate exposure (fig. 7).

When $100 \mu\text{M}$ ammonium persulphate was added to the organ bath, the NANC relaxations were reduced by $57.2 \pm 15.2\%$ ($p < 0.05$) in the control group, a reduction similar to that caused by in vivo inhalation. In the persulphate-exposed group, in vitro treatment with $100 \mu\text{M}$ ammonium persulphate did not cause a significant reduction in NANC relaxations ($12.8 \pm 22.3\%$ as compared with baseline). The baseline tone of the tracheal preparations (in both control and persulphate-exposed animals) was not affected by in vitro administration of ammonium persulphate.

Discussion

The present study provides the evidence that inhalation of ammonium persulphate at high concentrations impairs the nervous NANC inhibitory control in the

Fig. 7. Cumulative concentration-response curves to carbachol (1×10^{-8} to 3×10^{-6} M) in isolated whole trachea in control and persulphate-treated animals. Contractions were measured as maximum intratracheal pressure increase (Pa). In exposed animals, the contraction evoked by 3×10^{-6} M carbachol was greater than that observed in control animals. Data represent the mean $\pm$ SEM of 5 experiments. * $p < 0.05$.

guinea pig isolated trachea, as observed by using a sub-maximal response to EFS at 3 Hz. As observed previously [10], NO and CO, both having cGMP as a second messenger, participate as neurotransmitters in sub-maximal NANC tracheal relaxations, as shown by the inhibition caused by L-NAME and ZnPP-IX, respectively. Among the NO synthase inhibitors, L-NAME is one of the most widely used compounds to assess the nitrergic component of the NANC inhibitory response [30]. ZnPP-IX is a haem oxygenase-2 inhibitor that is used to assess the involvement of CO in functional studies. We are aware that the use of metalloporphyrins in CO research is not devoid of criticism owing to the effects of metalloporphyrins distinct from the inhibition of haem oxygenase, namely inhibition of NOS and soluble guanylyl cyclase, [31], but these effects were observed at ZnPP-IX concentration 10-fold higher than that used in our study. Conversely, our results show no effect of ZnPP-IX used alone at 10 μ M and allowed us to discriminate between the effects of haem oxygenase-2, NO synthase and soluble guanylyl cyclase inhibition. This question has been compre-

hensively discussed in a previous paper by our group [10].

In persulphate-exposed animals, the NANC relaxation to 3 Hz was approximately halved compared with controls. Nevertheless, the contribution of each inhibitory neurotransmitter to the NANC relaxation was not modified, suggesting a global reduction in the function of the intrinsic inhibitory innervation, which leads to a lower neurotransmitter release, or a faster inactivation of released neurotransmitters. The reduction in NANC relaxation cannot be attributed to a different basal tone of the tracheal smooth muscle or to a generalized impaired responsiveness to relaxing agents, as shown by the baseline tone and isoprenaline-induced relaxation data. The presence of a marked inflammatory infiltration (about 5 fold as compared with control), resembling that observed in natural asthma, confirmed the suitability of our animal model. Eosinophil infiltration of the airways, as observed in our histological preparations, is known to be a general feature of allergic asthma, that can also be observed in persulphate-induced occupational asthma [5].

Administration of ammonium persulphate in vitro caused a concentration-dependent dilation of guinea pig isolated trachea, through a mechanism mediated by NO [20]. The source of NO release was not identified, but epithelial NO [32] might be involved in that relaxing response. Conversely, under our experimental conditions (i.e., tracheal segments deprived of mucosa), we did not observe any change in basal tone. In the same paper [20], a hypothesis was proposed suggesting a potential dysregulation of NO-mediated responses as a cause of airway hyperreactivity due to a chronic exposure to oxidizing substances. Our data add experimental evidence to this hypothesis.

The mechanisms underlying persulphate-induced asthma include immunologic humoral and cellular events. Immunoglobulin E is known to participate in the sensitizing process, but its role has not been well defined yet [2, 5]. Mast cells are thought to be involved in the pathogenesis of persulphate-induced asthma by releasing a number of inflammatory mediators, including histamine, arachidonic acid derivatives, biogenic amines, chemoattractants, cytokines, growth factors and neuropeptides [13, 33]. Direct irritation and damage of the airway mucosa is also thought to play a role [34, 35].

Because cholinergic nerves are the dominant neural bronchoconstrictor pathway in animal and human airways [36], a part of our work was aimed at investigating whether cholinergic mechanisms were altered following ammonium persulphate inhalation. It is known that in-

flammatory mediators may lead to enhanced cholinergic neurotransmission in the airways due to facilitation of acetylcholine release in parasympathetic ganglia or from post-ganglionic nerve terminals [37, 38]. Furthermore, inhalation of well-known occupational agents causing asthma, such as formaldehyde [39] and diisocyanates [40], has been found to heighten airway smooth muscle responsiveness to acetylcholine or carbachol in the guinea pig isolated trachea. In our experimental model, ammonium persulphate exposure did not change the extent of neurogenic cholinergic tracheal contractions. The muscular response to exogenous carbachol was unaffected up to 1 μM , whereas the response to the maximal concentration of carbachol (3 μM) was significantly increased following ammonium persulphate exposure. This suggests that the muscular response to acetylcholine is not increased under sub-maximal nerve stimulation, but an exaggerated smooth muscle contraction can occur when large amounts of the neurotransmitter are released (or when high concentrations of an exogenous cholinergic agonist are added to the bath) as a consequence of the massive activation of cholinergic reflexes via afferents in or outside the airways [7].

The contractile response to exogenous histamine was not increased in the exposed animals. This indicates that ammonium persulphate inhalation does not affect the muscular responsiveness to histamine, another main bronchoconstrictor mediator released in immediate hypersensitivity processes. On the other hand, it is well known that in subjects with occupational asthma, the degree of airway responsiveness to histamine (or methacholine) is usually, but not invariably, increased [8].

In conclusion, our data show that exposure to ammonium persulphate at high concentrations impairs the ner-

vous NANC inhibitory control in the guinea pig airways. The two neurotransmitters identified in the NANC inhibitory system [10], namely NO and CO, both having cGMP as a common intracellular messenger, are still present after ammonium persulphate exposure and they contribute to the neurogenic relaxation in the same proportion observed in the airways from non-exposed animals. Therefore, ammonium persulphate exposure does not affect in particular the single NOS- or the HO-related pathway. The observed impairment of NANC inhibitory responses is likely due to a global reduction in the function of the intrinsic inhibitory innervation, either because of a lower release of inhibitory neurotransmitters, or to a faster inactivation of released neurotransmitters.

Though guinea pig and human airways differ in several aspects, the impairment of the NANC inhibitory control represents a novel pathogenetic hypothesis of persulphate-induced asthma, that should be taken into account when investigating the pathogenesis of this occupational respiratory disease. Moreover, the impairment of NANC inhibitory control, as a consequence of persulphate exposure, should also be investigated in occupational asthma due to other chemicals having both irritating and sensitizing properties (e.g., isocyanates and glutaraldehyde), as well as in irritant-induced asthma, a type of occupational disorder without a latency period [12, 26].

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References

- 1 Tarlo SM, Liss GM, Dias C, Banks DE: Assessment of the relationship between isocyanate exposure and occupational asthma. *Am J Ind Med* 1997;32:517–521.
- 2 Munoz X, Cruz MJ, Orriols R, Bravo C, Espuga M, Morell F: Occupational asthma due to persulfate salts: diagnosis and follow-up. *Chest* 2003;123:2124–2129.
- 3 Moscato G, Galdi E: Asthma and hairdressers. *Curr Opin Allergy Clin Immunol* 2006; 6:91–95.
- 4 Kopferschmitt-Kubler MC, Ameille J, Popin E, Calastreng-Crinquand A, Vervloet D, Bayeux-Dunglas MC, Pauli G; Observatoire National de Asthmes Professionnels Group: Occupational asthma in France: a 1-year report of the Observatoire National de Asthmes Professionnels project. *Eur Respir J* 2002;19: 84–89.
- 5 Moscato G, Pignatti P, Yacoub MR, Romano C, Spezia S, Perfetti L: Occupational asthma and occupational rhinitis in hairdressers. *Chest* 2005;128:3590–3598.
- 6 Canning BJ, Fischer A: Neural regulation of airway smooth muscle tone. *Respir Physiol* 2001;125:113–127.
- 7 Barnes PJ: Overview of neural mechanisms in asthma. *Pulm Pharmacol* 1995;8:151–159.
- 8 Mapp C, Boschetto P, Miotto D, De Rosa E, Fabbri LM: Mechanisms of occupational asthma. *Ann Allergy Asthma Immunol* 1999;83:645–664.
- 9 Dellabianca A, Faniglione M, De Angelis S, Tonini S, Balestra B, Colucci M, Cervio M, Clavanzani P, Chiocchetti R, De Giorgio R, Candura SM: Adenosine A1 and A3 receptor agonists inhibit nonadrenergic, noncholinergic relaxations in the guinea pig isolated trachea. *Respiration* 2009;78:75–83.

- 10 Dellabianca A, Sacchi M, Anselmi L, De Amici E, Cervio E, Agazzi A, Tonini S, Sternini C, Tonini M, Candura SM: Role of carbon monoxide in electrically induced non-adrenergic, non-cholinergic relaxations in the guinea-pig isolated whole trachea. *Br J Pharmacol* 2007;150:220–226.
- 11 Barnes PJ: Neuroeffector mechanisms: the interface between inflammation and neuronal responses. *J Allergy Clin Immunol* 1996; 98:S73–S81.
- 12 Mapp CE, Boschetto P, Maestrelli P, Fabbri LM: Occupational asthma. *Am J Respir Crit Care Med* 2005;172:280–305.
- 13 Blainey AD, Ollier S, Cundell D, Smith RE, Davies RJ: Occupational asthma in a hair-dressing salon. *Thorax* 1986;41:42–50.
- 14 Bardana EJ Jr: Occupational asthma and allergies. *J Allergy Clin Immunol* 2003;111: S530–S539.
- 15 Miura M, Ichinose M, Kimura K, Katsumata U, Takahashi T, Inoue H, Takishima T: Dysfunction of nonadrenergic noncholinergic inhibitory system after antigen inhalation in actively sensitized cat airways. *Am Rev Respir Dis* 1992;145:70–74.
- 16 Barnes PJ: Neurogenic inflammation and asthma. *J Asthma* 1992;29:165–180.
- 17 Barnes PJ, Liew FY: Nitric oxide and asthmatic inflammation. *Immunol Today* 1995; 16:128–130.
- 18 Belvisi MG, Stretton CD, Miura M, Verleden GM, Tadjikarim S, Yacoub MH, Barnes PJ: Inhibitory NANC nerves in human tracheal smooth muscle: a quest for the neurotransmitter. *J Appl Physiol* 1992;73:2505–2510.
- 19 Jartti T: Asthma, asthma medication and autonomic nervous system dysfunction. *Clin Physiol* 2001;21:260–269.
- 20 Mensing T, Marek W, Baur X: The influence of ammonium persulfate on guinea pig tracheal muscle tone: release of nitric oxide. *Pharmacol Toxicol* 1996;78:336–340.
- 21 Zosky GR, Sly PD: Animal models of asthma. *Clin Exp Allergy* 2007;37:973–988.
- 22 Pauluhn J, Mohr U: Experimental approaches to evaluate respiratory allergy in animal models. *Exp Toxicol Pathol* 2005;56:203–234.
- 23 Nials AT, Uddin S: Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Model Mech* 2008;1:213–220.
- 24 Merget R, Buenemann A, Kulzer R, Rueckmann A, Breitstadt R, Kniffka A, Kratisch H, Vormberg R, Schultze-Werninghaus G: A cross sectional study of chemical industry workers with occupational exposure to persulphates. *Occup Environ Med* 1996;53:422–426.
- 25 Mensing T, Marek W, Baur X: Modulation of airway responsiveness to acetylcholine by nitric oxide in a rabbit model. *Lung* 1997;175: 367–377.
- 26 Malo JL: Irritant-induced asthma and reactive airways dysfunction syndrome. *Can Respir J* 1998;5:66–67.
- 27 Tanihata S, Uchiyama T: Role of nitric oxide in nonadrenergic, noncholinergic relaxation of whole tracheal tube preparations isolated from guinea pigs. *Gen Pharmacol* 1996;27: 827–832.
- 28 D'Agostino G, Chiari MC, Grana E, Subissi A, Kilbinger H: Muscarinic inhibition of acetylcholine release from a novel in vitro preparation of the guinea-pig trachea. *Naunyn Schmiedeberg's Arch Pharmacol* 1990; 342:141–145.
- 29 Zygmunt PM, Hogestatt ED, Grundemar L: Light-dependent effects of zinc protoporphyrin IX on endothelium-dependent relaxation resistant to N omega-nitro-L-arginine. *Acta Physiol Scand* 1994;152:137–143.
- 30 Nevin BJ, Broadley KJ: Nitric oxide in respiratory diseases. *Pharmacol Ther* 2002;95: 259–293.
- 31 Grundemar L, Ny L: Pitfalls using metalloporphyrins in carbon monoxide research. *Trends Pharmacol Sci* 1997;18:193–195.
- 32 Bove PF, van der Vliet A: Nitric oxide and reactive nitrogen species in airway epithelial signaling and inflammation. *Free Radic Biol Med* 2006;41:515–527.
- 33 Pisati G, Baruffini A, Cirila AM: Asma professionale da polveri decoloranti per capelli: considerazioni su un caso clinico. *Med Tor* 1988;10:79–83.
- 34 Mensing T, Marek W, Raulf-Heimsoth M, Baur X: Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model. *Eur Respir J* 1998;12:1371–1374.
- 35 Munakata M, Huang I, Mitzner W, Menkes H: Protective role of epithelium in the guinea pig airway. *J Appl Physiol* 1989;66:1547–1552.
- 36 Barnes PJ: Neural control of human airways in health and disease. *Am Rev Respir Dis* 1986;134:1289–1314.
- 37 Barnes PJ: Modulation of neurotransmission in airways. *Physiol Rev* 1992;72:699–729.
- 38 Undem BJ, Myers AC, Weinreich D: Antigen-induced modulation of autonomic and sensory neurons in vitro. *Int Arch Allergy Appl Immunol* 1991;94:319–324.
- 39 Swiecichowski AL, Long KJ, Miller ML, Leikauf GD: Formaldehyde-induced airway hyperreactivity in vivo and ex vivo in guinea-pigs. *Environ Res* 1993;61:185–199.
- 40 Marek W, Potthast J, Marczynski B, Mensing T, Baur X: Subchronic exposure to diisocyanates increases guinea pig tracheal smooth muscle responses to acetylcholine. *Respiration* 1999;66:156–161.