Chronic exposure to ozone causes tolerance to airway hyperresponsiveness in guinea pigs: lack of SOD role

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Vargas, Mario H., Laura Romero, Bettina Sommer, Pedro Zamudio, Pascal Gustin, and Luis M. Montaño. Chronic exposure to ozone causes tolerance to airway hyperresponsiveness in guinea pigs: lack of SOD role. J. Appl. Physiol. 84(5): 1749–1755, 1998.—Tolerance to respiratory effects of O3 has been demonstrated for anatomic and functional changes, but information about tolerance to O3-induced airway hyperresponsiveness (AHR) is scarce. In guinea pigs exposed to air or O3 (0.3 parts/million, 4 h/day, for 1, 3, 6, 12, 24, or 48 days, studied 16–18 h later), pulmonary insufflation pressure changes induced by intravenous substance P (SP, 0.032–3.2 µg/kg) were measured, then the animals were subjected to bronchoalveolar lavage (BAL). Bronchial rings with or without phosphoramidon were also evaluated 3 h after air or a single O3 exposure. O3 caused in vivo AHR with or without phosphoramidon were also evaluated 3 h after air or a single O3 exposure. O3 caused in vivo AHR (increased sensitivity) to SP after 1, 3, 6, 12, and 24 days of exposure compared with control. However, after 48 days of exposure, O3 no longer caused AHR. Total cell, macrophage, neutrophil, and eosinophil counts in BAL were increased in most O3-exposed groups. When data from all animals were pooled, we found a highly significant correlation between degree of airway responsiveness and total cells (r = 0.55), macrophages (r = 0.54), neutrophils (r = 0.47), and eosinophils (r = 0.53), suggesting that airway inflammation is involved in development of AHR to SP. Superoxide dismutase (SOD) levels in BAL fluids were increased (P < 0.05) after 1, 3, 6, and 12 days of O3 exposure and returned to basal levels after 24 and 48 days of exposure. O3 failed to induce hyperresponsiveness to SP in bronchial rings, and phosphoramidon increased responses to SP in air- and O3-exposed groups, suggesting that neutral endopeptidase inactivation was not involved in O3-induced AHR to SP in vivo. We conclude that chronic exposure to 0.3 ppm O3, a concentration found in highly polluted cities, resulted in tolerance to AHR to SP in guinea pigs by an SOD-independent mechanism.

A great number of studies have demonstrated that acute ozone (O3) exposure can induce many deleterious effects on the respiratory system in humans and animals, including anatomic and ultrastructural changes (1, 4, 20), a decrease in expiratory flows and volumes (12, 14), and airway hyperresponsiveness (7, 24). Nevertheless, in an increasing number of published works, respiratory alterations induced by an acute exposure to O3 have been found to diminish or disappear when this exposure is repeated for several days; i.e., a phenomenon of tolerance is developed. This tolerance has been demonstrated for the O3-induced anatomic changes (21) as well as for the deleterious effect on lung flows and volumes (11, 30); however, very little information is available about the development of tolerance to airway hyperresponsiveness (10). This last issue is important, because airway hyperresponsiveness is a functional feature of asthmatic patients and because the number of emergency room visits by asthmatic patients increases after episodes of high urban O3 pollution (22). Thus, because exposure to O3 seems to be a factor that worsens asthma symptoms in patients living in polluted cities (8), it is important to investigate the development and mechanisms of tolerance to O3-induced airway hyperresponsiveness.

In a previous work we exposed guinea pigs to 0.15–1.2 parts/million (ppm) O3 and tested airway responsiveness with acetylcholine, histamine, and substance P (23). We found that substance P was the most sensitive mediator to demonstrate O3-induced airway hyperresponsiveness, since hyperresponsiveness to substance P was evident after exposure to O3 concentrations as low as 0.3 ppm, a concentration that can be reached in very highly polluted cities, whereas histamine showed such an effect only at 1.2 ppm O3 and acetylcholine did not result in hyperresponsiveness at any O3 concentration. Thus in the present study we decided to investigate whether this O3-induced airway hyperresponsiveness to substance P persists after prolonged O3 exposure and to correlate the results with the inflammatory changes. In addition, because superoxide anion has been involved in the development of O3-induced airway hyperresponsiveness (16, 26, 28), we also evaluated the changes in superoxide dismutase (SOD) levels during this repetitive O3 exposure.

MATERIALS AND METHODS

Animals and O3 exposure. Male Hartley guinea pigs (500–600 g) bred in our institutional animal facilities (filtered conditioned air, 21 ± 1°C, 50–70% humidity, sterilized bed) and fed Purina pellets supplemented with disinfected fresh alfalfa and sterilized water were used. An air filter (Heaven, AllerMed) was used to ensure that all the animals were maintained in an environment with a minimum O3 concentration (<0.015 ppm). The animals remained under these conditions until the study. The project was approved by our Animal Care Committee, and the experiments were conducted in accordance with the “Guiding Principles in the Care and Use of Animals” of the American Physiological Society.

Guinea pigs were exposed to 0.3 ± 0.004 (SE) ppm O3 for 4 h/day for 1, 3, 6, 12, 24, or 48 days (n = 6–7/group). In the last three periods, animals were exposed to O3 for 6 days/wk. All animals were studied 16–18 h after the last O3 exposure.

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Because animal groups were exposed to O\textsubscript{3} for different periods of time, to avoid biases due to differences in age, O\textsubscript{3} exposure began at an earlier age in guinea pigs exposed for longer periods than in those exposed for shorter periods. In this way, the age of all the animals was similar at the time of the study. Control animals (n = 6) were exposed for 4 h to filtered airflow in a similar chamber 16–18 h before the study. We confirmed that at the time of the study the body weights of the animals were not different among the groups: 556 ± 21.6 g for the control group and 548.7 ± 24.4, 540.5 ± 14.6, 574.0 ± 22.0, 563.7 ± 15.3, 572.5 ± 28.1, and 590.2 ± 13.3 g for 1, 3, 6, 12, 24, and 48 days of O\textsubscript{3} exposure, respectively (P = 0.70, by ANOVA). O\textsubscript{3} was produced by passing a constant airflow (3 l/min) through an ozonizer (Puraqua-V, Purificadores Eléctricos por Ozono) into which an electric arc converted air to O\textsubscript{3}. The O\textsubscript{3} concentration was regulated by modifying the voltage delivered to the ozonizer. O\textsubscript{3} inside the acrylic chamber was continuously monitored by an ultraviolet O\textsubscript{3} analyzer (model 1008 PC, Dynotest). The O\textsubscript{3} concentration was steady for 1, 3, 6, 12, 24, and 48 days of O\textsubscript{3} exposure, respectively (P = 0.21, 6.03, 0.33, 6.03, 0.20, 5.87 ± 0.28, 6.67 ± 0.10, 6.53 ± 0.28, and 6.18 ± 0.33 ml from the animals exposed to O\textsubscript{3} for 1, 3, 6, 12, 24, and 48 days, respectively (P = 0.29, by ANOVA). Total recovered fluid was immediately centrifuged for 10 min at 5000 g, 4°C, the cell pellet was resuspended in 1 ml of PBS, and total cells were counted by use of a Neubauer hemocytometer. Smears stained with Romanowsky stain for differential cell count were obtained with a slide stainer (7120 Aerospray, Wescor). Cell counts were expressed as number of cells per milliliter of BAL fluid. Cell viability > 80% was confirmed by the trypan blue exclusion technique in every BAL. All smears were coded, and cells were counted by a pathologist who did not know the code.

SOD measurement. Extracellular SOD in BAL fluids (after cell removal) was measured through competitive ELISA by the biotin-streptavidin-peroxidase method. Guinea pig SOD was isolated from red blood cells by the following method: erythrocytes were obtained from healthy guinea pigs, washed twice with PBS (pH 7.2) with proteolysis inhibitors (trypsin inhibitor, aprotinin, phenylmethylsulfonyl fluoride) and thimerosal, lysed by hypotonic shock, and reconstituted with PBS. The suspension was sequentially passed through a sterilized glass filter, a Whatman no. 1 paper, and a 0.22-mm Millipore membrane. From this solution, SOD was purified using affinity chromatography with cyanogen bromide-activated sepharose 4B coupled to human SOD monoclonal antibodies. Purified SOD was passed through an affinity chromatogram column with sepharose 4B coupled to protein A to eliminate possible contamination with Fc fraction unspecifically coupled to SOD. The SOD concentration was calculated by measuring the amount of purified protein by the Lowry method. ELISA plaques were activated with the purified guinea pig SOD. The BAL fluid sample and biotin-labeled human SOD monoclonal antibodies were mixed, added to the ELISA plaques, and incubated at room temperature for 1 h in an automatic shaker. Plaques were submitted to 10 washing cycles with a mixture of PBS, Tween 20, and Triton X-100. After a 30-min incubation with streptavidin-peroxidase, plaques were washed again 10 times and incubated with o-phenylenediamine and H\textsubscript{2}O\textsubscript{2} in citrate buffer (pH 4.3). The reaction was stopped with 5% sulfuric acid (vol/vol), and the sample was read at 492 nm using an ELISA lecturer (Multiskan MS model MCC/340, Labsystems).

In vitro studies. A separate group of animals was acutely exposed to air or O\textsubscript{3} (0.3 ppm for 4 h) and immediately anesthetized with pentobarbital and exsanguinated. The
respiratory tract was carefully dissected and cleaned of connective tissue. Two main bronchi rings were obtained from every animal. Each tissue was hung in a 5-ml organ bath between two hooks inserted into the lumen. One of the hooks was attached to an isometric transducer (model UC3, Gould Statham) by a 4-0 silk thread. The second hook was made of platinum and acted as an anchor by keeping the ring fixed to a Plexiglas rod. The poles for the electrical stimuli were the second platinum hook and a platinum wire fastened to the Plexiglas rod. Tissues were located between these two poles.

The Krebs solution in the organ baths (in mM: 120 NaCl, 4.77 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, 2.5 CaCl2, and 11 glucose) was maintained at 37°C and bubbled with 5% CO2-95% O2 (pH 7.4). Tissues were placed under a resting tension of 1 g, washed with fresh Krebs solution, and maintained in these conditions for 30 min. Isometric tension was recorded on a polygraph (model R612, Beckman).

An electrical field stimulation (10-s trains, 2 ms, 100 V, 16 Hz) was delivered to each preparation to verify tissue viability. A cumulative concentration-response curve to 10-10-10-4 M substance P was determined for every bronchus. Contractile responses to substance P were expressed as a percentage of the maximum contraction previously elicited by 60 mM KCl in the same preparation. Determination of concentration-response curves began 3.35 ± 0.29 h after the air or O3 exposure. In some bronchial tissues, phosphoramidon (10-5 M), a known neutral endopeptidase (NEP) inhibitor, was added to the organ bath 10 min before the concentration-response curve to substance P.

Drugs. Reconstituted aprotinin from bovine lung, type II-S trypsin inhibitor from soybean, phenylmethylsulfonyl fluoride, thimerosal, substance P acetate salt, phosphoramidon sodium salt, and monoclonal human SOD antibodies were purchased from Sigma Chemical (St. Louis, MO).

Data analysis. The negative logarithm of the 50% effective dose (−log ED50) was calculated for every dose-response curve by straight-line regression plotting logarithm of the dose vs. the probit-transformed response. Statistical evaluation was done using one-way ANOVA followed by Dunnett’s test to compare the control group with each experimental group. To determine the possible role of the cells recovered in BAL fluid in the development of airway hyperresponsiveness, statistical associations between cell populations and the degree of airway responsiveness were made by Pearson’s correlation coefficient. In vitro concentration-response curves were evaluated by analysis of covariance by plotting the 1/log concentration of substance P on the x-axis against the bronchial contraction expressed as the percentage of 60 mM KCl on the y-axis. In this last analysis, multiple comparisons were corrected by Bonferroni’s method. Statistical significance was set at two-tailed P < 0.05. Values are means ± SE.

RESULTS

Compared with the control group, leftward displacements of the dose-response curve for substance P were observed after O3 exposure for 1, 3, 6, 12, and 24 days (Fig. 2, left), reaching statistical significance (P < 0.01) when evaluated by the −log ED50 (mg/kg): 3.2059 ± 0.0535 (control) vs. 3.6184 ± 0.0482, 3.6325 ± 0.0554, 3.9247 ± 0.0457, 3.6218 ± 0.0371, and 3.7773 ± 0.0687, respectively. However, after 48 days of exposure, O3 no longer caused airway hyperresponsiveness, since the −log ED50 returned to control levels (3.3291 ± 0.0464; Fig. 2, right). In separate groups of control animals (n = 5) and guinea pigs exposed to O3 for 1 day (n = 5), we found that O3 did not modify the basal values of mean arterial pressure or the decrement in blood pressure induced by each substance P dose (data not shown).

All experimental groups exposed to O3 showed a significant increase (P < 0.01) in the total cell number compared with the control group (Fig. 3). In addition, almost all exposed groups showed a significant increase (P < 0.01) in the number of macrophages, neutrophils, and eosinophils. Conversely, the number of lymphocytes increased only in the groups exposed to O3 for 24 and 48 days (P < 0.01). Because each BAL was performed after substance P administration, in a separate group of guinea pigs (n = 4) exposed to O3 for 1 day, we confirmed that O3 was able to induce the same inflammatory response, regardless of the administration of substance P (data not shown).

When data from all the animals were pooled, a highly significant correlation was observed between the degree of airway responsiveness and the total cell (r = 0.55, P < 0.0002), macrophage (r = 0.54, P < 0.0002),
neutrophil \( (r = 0.47, P < 0.002) \), and eosinophil \( (r = 0.53, P < 0.0005) \) counts (Fig. 4). Moreover, when values from the animals exposed to \( \text{O}_3 \) for 48 days were removed from the analysis, the correlation coefficients showed a further increase \((r = 0.69, P < 10^{-5}; r = 0.65, P < 10^{-4}; r = 0.63, P < 10^{-3}; r = 0.70, P < 10^{-3}, \) respectively). No correlation was found between airway responsiveness and lymphocyte counts.

SOD levels in BAL fluid of the control group \((0.29 \pm 0.061 \text{ mg/ml}) \) increased \((>30\text{-fold}, P < 0.05) \) after 1 day of \( \text{O}_3 \) exposure \((8.98 \pm 3.19 \text{ mg/ml}) \); further increased \((>60\text{-fold}, P < 0.01) \) after 3, 6, and 12 days \((17.62 \pm 3.17, 15.90 \pm 3.81, \text{ and } 15.40 \pm 2.81 \text{ mg/ml}, \) respectively\); and then returned to basal levels after 24 and 48 days of exposure \((0.98 \pm 0.08 \text{ and } 0.48 \pm 0.08 \text{ mg/ml}, \) respectively; Fig. 5).

To simplify the evaluation of the relationships among airway responsiveness, total cell count, and SOD concentration, all these variables are plotted together in Fig. 6.

In the in vitro studies using bronchial preparations \((n = 6 \text{ for each group}) \), we found that a single acute \( \text{O}_3 \) exposure did not modify the concentration-response curve to substance P, at least at 3.35 h after conclusion of the exposure. By contrast, in air- and \( \text{O}_3 \)-exposed tissues, phosphoramidon produced a notable leftward displacement of the concentration-response curves to substance P (Fig. 7), which was highly statistically significant \((P < 10^{-6}) \) when evaluated by analysis of covariance (Table 1).

Fig. 3. Changes in profiles of cells recovered in bronchoalveolar lavage from guinea pigs exposed to air (0 days) or to 0.3 ppm \( \text{O}_3 \) for 4 h/day for 1, 3, 6, 12, 24, and 48 days. Bars, average of 6–7 animals; error bars, SE. *\( P < 0.05; **P < 0.01. \)

Fig. 4. Correlations between airway responsiveness to substance P and total cells, macrophages, and eosinophils recovered in bronchoalveolar lavage from guinea pigs exposed to air or to 0.3 ppm \( \text{O}_3 \) for 4 h/day for 1, 3, 6, 12, 24, and 48 days.

Fig. 5. Effect of exposure to air (0 days) or to 0.03 ppm \( \text{O}_3 \) for 4 h/day for 1, 3, 6, 12, 24, and 48 days on superoxide dismutase concentrations in bronchoalveolar lavage from guinea pigs. Bars, average of 6–7 animals; error bars, SE. *\( P < 0.05; **P < 0.01. \)
exposure. Phos, 10$^{-5}$ M phosphoramidon. Symbols, average of 6 preparations.

**DISCUSSION**

In this work we found that O$_3$ (0.3 ppm, 4 h/day) induced airway hyperresponsiveness to substance P after 1, 3, 6, 12, and 24 days of exposure. However, this hyperresponsiveness was no longer present after 48 days of O$_3$ exposure, i.e., a phenomenon of tolerance to the O$_3$ effect was developed.

The O$_3$-induced airway hyperresponsiveness to substance P was described some years ago in vitro and in vivo (7, 23, 31), but its mechanisms of production are still controversial. In the present study we found a sustained increase in the number of inflammatory cells such as eosinophils, neutrophils, and macrophages during all 48 days of O$_3$ exposure as well as an increase in the SOD concentration during the first 12 days of the study. Because SOD is an enzyme specifically induced by its substrate the superoxide anion, this high level of SOD implies that increasing amounts of superoxide anions were produced during the repetitive O$_3$ exposures. It has been demonstrated that inflammatory cells are a major source of superoxide anion, and even this product has been considered a marker of cell activation (2, 17). Reactive oxygen species, including superoxide anion, have been implicated in the acute O$_3$-induced (2–3 ppm) airway hyperresponsiveness to methacholine, histamine, and bradykinin in guinea pigs, rats, and cats (16, 26, 28). Thus in the present work it would be possible to speculate that superoxide anion released by inflammatory cells could also be involved in the development of O$_3$-induced airway hyperresponsiveness to substance P. In addition, inflammatory cells are able to release a number of mediators such as thromboxane A$_2$ and leukotriene C$_4$, which can directly induce hyperresponsiveness in the airway smooth muscle (3). In support of the possible role of inflammation in this phenomenon, we found a close relationship between total cells, macrophages, neutrophils, and eosinophils recovered in the lavage fluid and airway responsiveness, at least during the first 24 days.

In a recent study (23) in guinea pigs, in which we used the same methodological procedures as in the present study, we found that exposure to 0.3 ppm O$_3$ caused airway hyperresponsiveness to substance P but not to histamine or acetylcholine. That study and the present results suggest that the increment in superoxide anion and the inflammation may not be sufficient to induce nonspecific airway hyperresponsiveness and that an additional factor is acting to promote specific airway hyperresponsiveness to substance P. In this context, it has been proposed (19, 31) that, in guinea pigs, oxidative inactivation of NEP, the main enzyme capable of inactivating substance P, may represent a major mechanism of the airway hyperresponsiveness to this agonist developed shortly after O$_3$ exposure. Muralis et al. (19) corroborated this NEP inactivation by a biochemical assay performed 30 min after exposure to 3 ppm O$_3$. Nevertheless, this mechanism seems not to be present in our study. If the airway hyperresponsiveness to substance P that we observed in vivo was due to a putative NEP inactivation, then such hyperresponsiveness should also be manifested in vitro conditions. In this sense, in a recent study using guinea pigs exposed to O$_3$, in the same conditions as in the present work, we found no hyperresponsiveness to substance P in bronchial rings in organ baths 18 h after O$_3$ exposure (25), which suggests that NEP was not affected by O$_3$. To corroborate this finding, we now investigated the possible effects of O$_3$ on NEP activity after a shorter (3 h) exposure to O$_3$. We found similar results; i.e., O$_3$ did not modify the bronchial responsiveness to substance P. Moreover, in experiments with phosphoramidon, an inhibitor of NEP, animals exposed to air and O$_3$ demonstrated a similar noteworthy increase in the responses to substance P. Taken together, these results point out that NEP inactivation is not a mechanism involved in the O$_3$-induced hyperresponsiveness to SP, at least at the concentration (0.3 ppm) used by us.

Some additional mechanisms could potentially be involved in the O$_3$-induced airway hyperresponsiveness to substance P and deserve further evaluation. For example, we recently found that O$_3$ enhances the bronchial nonadrenergic noncholinergic response to electrical field stimulation by introducing an atropine-sensitive component (25). Therefore, another possible specific mechanism induced by O$_3$ may be an enhanced ability of substance P to release acetylcholine from

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**Table 1. Analysis of covariance of bronchial responses to substance P**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plotted Points</th>
<th>$r$</th>
<th>Slope</th>
<th>y-Axis Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>29</td>
<td>0.86</td>
<td>557.75</td>
<td>−64.51</td>
</tr>
<tr>
<td>Air + phosphoramidon</td>
<td>41</td>
<td>0.85</td>
<td>674.74</td>
<td>−42.88*</td>
</tr>
<tr>
<td>O$_3$</td>
<td>30</td>
<td>0.85</td>
<td>601.02</td>
<td>−73.07</td>
</tr>
<tr>
<td>O$_3$ + phosphoramidon</td>
<td>41</td>
<td>0.73</td>
<td>560.69</td>
<td>−20.02†</td>
</tr>
</tbody>
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Bronchi of O$_3$-exposed groups were obtained from animals exposed to 0.3 ppm O$_3$ for 4 h. Phosphoramidon ($10^{-5}$ M) was added 10 min before substance P concentration-response curve. Points correspond to 1/Log concentration of substance P in x-axis plotted against bronchial contractions expressed as percentage of 60 mM KCl response. Plotted points, responses to all concentrations of substance P in each group; $r$, Pearson’s correlation coefficient. * $P < 10^{-6}$ compared with control (air) group; † $P < 10^{-6}$ compared with O$_3$ group.
cholinergic nerves (13). Additionally, it has been demonstrated that substance P acts on some vascular beds, causing endothelial leakiness and release of NO and prostanooids from endothelial cells (5, 6, 9). Thus a possible effect of O₃ on an SP-sensitive vascular component may also play a role in the increased sensitivity to SP after O₃ exposure.

One of the most remarkable findings of our study was that at 48 days of O₃ exposure the airway hyperresponsiveness to substance P disappeared; i.e., O₃-induced airway hyperresponsiveness underwent an adaptive phenomenon (tolerance) after chronic exposure to this agent. As mentioned above, one of the mechanisms probably involved in the development of acute O₃-induced airway hyperresponsiveness is the production of superoxide anion. Thus we investigated the possible role of SOD in the development of the tolerance to the O₃ effect. In the present work the remarkable 30-fold increase in SOD levels from the 1st day of O₃ exposure raises the possibility that this antioxidant mechanism constitutes one of the responses immediately activated to counteract the deleterious effect of reactive oxygen species released by O₃-induced inflammation, as has been demonstrated for glutathione in the rat (27).

However, SOD could not be responsible for the tolerance to the effect of O₃ after 48 days of exposure, since SOD concentrations dramatically returned to basal levels after 24 days of O₃ exposure, when airway hyperresponsiveness was still present. In this context, other antioxidant compounds have been reported to be increased after O₃ exposure. Kodavanti et al. (15) found that total glutathione and uric acid, two important antioxidant mechanisms, increased after 1 wk of exposure (23 h/day) to 0.8 but not 0.2 ppm O₃ in guinea pig BAL fluids and lung homogenates. They also found that ascorbate was also increased after exposure to 0.2 ppm O₃. Similarly, Tepper et al. (27) observed a slight increase in ascorbate levels in rat lung homogenates after 3 days of exposure to 0.5 ppm O₃. Thus it is possible that antioxidant mechanisms other than SOD are involved in the development of tolerance to long-term O₃ exposure. Moreover, after 12 days of exposure, this hypothetical increase in antioxidant mechanisms must be high enough to explain the reduction in the already increased SOD levels. Studies are underway to define the role of these antioxidants in the tolerance to O₃.

An interesting finding was that at 48 days of O₃ exposure the close correlation between airway responsiveness and inflammatory cells was no longer present, since airway hyperresponsiveness to substance P disappeared, despite the persisting increment in BAL cell numbers. As mentioned above, during the first 24 days of O₃ exposure the inflammatory cells are probably involved in the induction of hyperresponsiveness through the release of superoxide anion and/or other mediators. Thus at 48 days of O₃ exposure the dissociation between inflammation and hyperresponsiveness may suggest that at this time inflammatory cells are still under the influence of chemoattractant factors but that they have lost their state of activation with a consequent diminution of their mediator release. This hypothesis is in full agreement with the notable diminution of the SOD levels, implying a reduced release of superoxide anion by these cells. Finally, additional mechanisms such as downregulation of receptors and altered production of NO and/or excitatory or inhibitory prostanooids could be involved in the tolerance phenomenon and deserve further investigation.

In conclusion, our results demonstrate that long-term exposure to 0.3 ppm O₃, a concentration that can be found in the atmosphere of highly polluted cities, induced tolerance to the airway hyperresponsiveness to substance P through a mechanism different from SOD induction.

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