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Ozone at high-pollution urban levels causes airway hyperresponsiveness to substance P but not to other agonists

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Abstract

Ozone (O_3) causes airway hyperresponsiveness, but few studies have evaluated this effect at urban concentrations. In this work dose-response curves to intravenous acetylcholine, histamine or substance P were performed in guinea pigs with or without previous exposure to O_3 (0.15, 0.3, 0.6 or 1.2 ppm for 4 h, 16-18 h before the studies). We found airway hyperresponsiveness to histamine, but not to acetylcholine, only after 1.2 ppm O_3. By contrast, airway hyperresponsiveness to substance P was developed at O_3 levels encountered in highly-polluted cities (0.3 ppm). These results suggest that excitatory non-adrenergic non-cholinergic responses could be affected by air pollution, and that substance P is a useful pharmacological tool for evaluating the airway hyperresponsiveness induced by low O_3 concentrations. © 1997 Elsevier Science B.V.

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1. Introduction

In the last decade O_3 concentrations in big cities have become an increasing concern due to the high levels found in their atmospheres. One of the most remarkable examples is Mexico city, where O_3 concentrations above Mexican air quality standards (0.11 ppm) are found almost every day of the year, frequently reaching levels over 0.3 ppm (33 days in 1992, 8 days in 1993, and 3 days in 1994, Mexico City Air Monitoring Network files). Recent epidemiological studies have suggested that urban pollution with O_3 causes an increased number of hospital admissions or emergency room visits of asthma patients (Burnett et al., 1994; Romieu et al., 1995). This could be explained by the deleterious

effect of O_3 on airway caliber or by its potentiation effect on airway response to allergens (Koren and Bromberg, 1995). Additionally, it is well known that experimental O_3 inhalation induces airway hyperresponsiveness both in humans (Holtzman et al., 1979; Seltzer et al., 1986) and animals (Gordon and Amdur, 1980; Campos et al., 1992). Nevertheless, most of these studies are intended to investigate the nature of the airway hyperresponsiveness phenomenon, and thus they use O_3 concentrations not related to urban pollution. Although some human studies have demonstrated airway hyperresponsiveness after O_3 concentrations as low as 0.08 or 0.12 ppm (McDonnell et al., 1991; Folinsbee et al., 1994), as far as we know there is a lack of animal models which can develop in vivo airway hyperresponsiveness after such low O_3 concentrations.

Thus, the aim of the present study was to evaluate the effect of several O_3 concentrations, including those encountered in polluted cities, on the airway respon-

siveness to histamine, acetylcholine and substance P in guinea pigs.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (500-600 g) bred in conventional conditions (filtered conditioned air, $21 \pm 1°C$, 50-70% humidity, sterilized bed) and fed with Purina pellets supplemented with disinfected fresh alfalfa and sterilized water were used. All the animals were placed in an environment with a minimum O_3 concentration (< 0.015 ppm O_3 by using a Heaven air-filter, Aller-Med., USA) before they were born and remained there until the study began.

2.2. Ozone exposure

Guinea pigs were exposed to 0.15, 0.3, 0.6, or 1.2 ppm O_3 for 4 h, and were studied 16-18 h later. The variation coefficient for these O_3 concentrations was approximately 7.5%. O_3 was produced by passing a constant airflow (3 l/min) through an ozonizer (Puraqua-V, Purificadores Eléctricos por Ozono, México) into which an electrical arc converted air to O_3. The O_3 concentration was regulated by modifying the voltage delivered to the ozonizer. O_3 inside the acrylic exposure chamber ($32 \times 48 \times 73$ cm) was continuously monitored by an ultraviolet O_3 analyzer (model 1008 PC, Dasibi Environmental, USA) connected to the chamber through Tygon tubes. Control animals were exposed to filtered airflow in similar chambers.

2.3. Pulmonary insufflation pressure

Animals were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and the depth of anesthesia maintained with hourly administration of additional pentobarbital (about 9 mg/kg, i.v.). Trachea was cannulated and mechanically ventilated (Harvard Apparatus, model 50-1700, England) with a tidal volume of 10 ml/kg and 48 breaths/min. Jugular vein and left carotid artery were cannulated for administration of drugs and arterial pressure recording through a Beckman 4-327-0129 transducer, respectively. Each animal received pancuronium bromide (0.06 mg/kg, i.v.) to avoid spontaneous respiratory movements.

Pulmonary insufflation pressure (PIP) was measured through a bronchospasm transducer (Ugo Basile, model 7020, Italy) connected to a collateral arm of the endotracheal tube. The increase in PIP was evaluated as the percentage of maximum obstruction obtained with artificial transient occlusion of the endotracheal tube. Although PIP is a less sensitive method than the more advanced ones (specific airway resistance, dynamic compliance, etc.), it is appropriate for estimation of bronchoconstriction in guinea pigs (Martling et al., 1984).

2.4. Dose-response curves

All guinea pigs received noncumulative increasing i.v. doses of acetylcholine (0.032 to 3.2 μg/kg), histamine (0.01 to 1.8 μg/kg) or substance P (0.0056 to 5.6 μg/kg) at 10 min intervals. Only one dose-response curve was made for each animal.

2.5. Drugs

Acetylcholine chloride, histamine dihydrochloride and substance P acetate salt (Sigma) were dissolved in 0.9% saline solution.

2.6. Data analysis

− Log effective dose 50% ($- \log ED_{50}$) was calculated in 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 analysis of variance, followed by Dunnett's test to compare control animals vs. each experimental group. Statistical association between O_3 concentration and $- \log ED_{50}$ was measured by Pearson's correlation coefficient. Statistical significance was set at two-tailed $p < 0.05$. Data in the text and figures are expressed as mean $\pm$ S.E.M.

3. Results

Dose-response curves to acetylcholine were not statistically modified after acute exposure to any O_3 concentration (0.3, 0.6 and 1.2 ppm; Fig. 1 (A) and Table 1). Accordingly, there was no correlation between O_3 concentration and acetylcholine $- \log ED_{50}$ ($r = 0.35$).

In relation to histamine, only the exposure to high O_3 concentration (1.2 ppm) induced leftward displacement of the dose-response curve to this agonist (Fig. 1 (B)) large enough to achieve statistical significance when evaluated through the $- \log ED_{50}$ ($p < 0.01$, Table 1). Although guinea pigs exposed to lower O_3-concentrations (0.15, 0.3, 0.6 ppm) showed leftward displacements of histamine dose-response curves, these changes were devoid of statistical significance. Nevertheless, pooling all of the animals there was a high correlation between the degree of airway reactivity and the inhaled O_3 concentration ($r = 0.66$, $p < 0.0005$).

Finally, dose-response curves to substance P were shifted to the left when guinea pigs were exposed to O_3 (Fig. 1 (C)). Compared to the control group, these
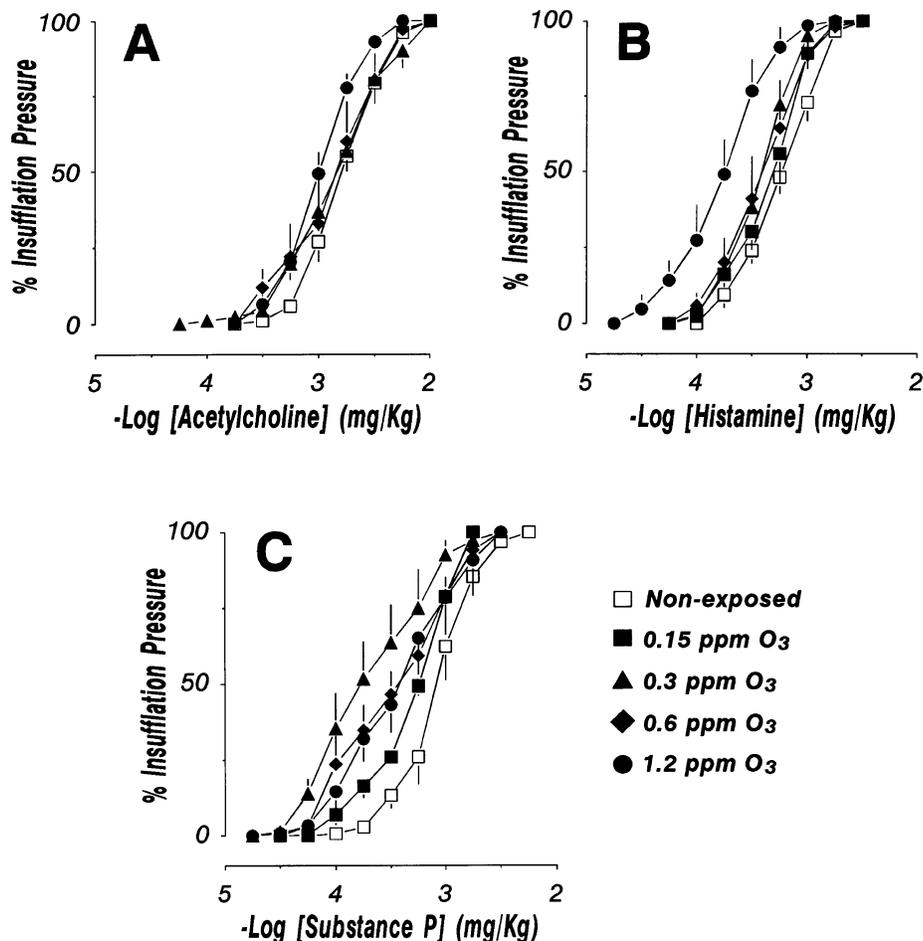
Fig. 1. Effect of exposure during 4 h to several ozone concentrations on the guinea pig airway responsiveness to acetylcholine (A), histamine (B) and substance P (C). Ozone exposure was performed 16–18 h previous study. Non-cumulative dose-response curves were performed through intravenous administration of the agonists at 10-min intervals.

displacements achieved statistical significance in the case of 1.2, 0.6 ($p < 0.05$) and 0.3 ppm ($p < 0.01$) groups, but not in the 0.15 ppm group (Table 1). Airway responsiveness ($-\log ED_{50}$) and O_3 concentration in all guinea pigs receiving substance P showed statistical correlation ($r = 0.40$, $p < 0.05$).

4. Discussion

Although O_3 has been extensively used as an irritating agent to produce models of airway hyperresponsiveness (Easton and Murphy, 1967; Murlas and Roum, 1985; Campos et al., 1992), most of these studies have used high-level O_3 exposures that are never found in the environment. Thus, the relevance of these studies in relation to air pollution phenomenon is questionable.

We used several O_3 concentrations, including those usually found in most polluted cities such as Mexico City, in order to elucidate the threshold concentration which could produce an increase in airway responsive-

ness in guinea pigs. Moreover, since mechanisms for airway hyperresponsiveness could vary according to the substance used to test the airway reactivity, we used three different drugs. We explored whether O_3 causes hyperresponsiveness to the neurotransmitter of cholinergic system (acetylcholine), to one of the main autacoids for the allergic bronchoconstriction in guinea pigs (histamine) and to a neuropeptide of the excitatory non-adrenergic non-cholinergic system (substance P). Our results showed that O_3 induced airway hyperresponsiveness to histamine and substance P, but not to acetylcholine.

In relation to the acetylcholine challenge, previous studies in guinea pigs (Murlas and Roum 1985) have found muscarinic airway hyperresponsiveness after exposure to high O_3 concentrations (3 ppm, 2 h). Therefore, our finding that O_3 did not cause airway hyperresponsiveness to acetylcholine could be due to the lower O_3 concentrations used by us. This assumption is in agreement with the results obtained by Nishikawa et al. (1990) who found airway hyperrespon-

Table 1
Effect of different ozone concentrations on the in vivo guinea pig airway responsiveness to acetylcholine, histamine and substance P

Ozone concentration (ppm)	n	Acetylcholine $-\log ED_{50}$ (mg/kg)	n	Histamine $-\log ED_{50}$ (mg/kg)	n	Substance P $-\log ED_{50}$ (mg/kg)
Non-exposed	6	2.7675 ± 0.0456	8	3.2525 ± 0.0533	6	3.0869 ± 0.0726
0.15			6	3.3521 ± 0.0536	5	3.2360 ± 0.0578
0.3	7	2.8152 ± 0.0860	6	3.4011 ± 0.0815	6	3.7176 ± 0.1365**
0.6	8	2.9024 ± 0.1194	6	3.4317 ± 0.1106	6	3.4574 ± 0.1012*
1.2	6	2.9878 ± 0.0349	7	3.7909 ± 0.1196**	5	3.5410 ± 0.0385*

* $p < 0.05$; ** $p < 0.01$ with respect to their respective non-exposed control (Dunnett's test).

siveness to methacholine in guinea pigs immediately after exposure to 1 ppm O_3 for 30 min, but this response disappeared after 5 h post-exposure. Since our study was performed 18 h after O_3 exposure, we were not able to find this early muscarinic hyperresponsiveness described by these authors. In summary, our results suggest that O_3 concentrations found in polluted cities are not high enough to produce enhanced airway reactivity to muscarinic agonists.

Airway hyperresponsiveness to histamine was found only at the highest O_3 concentration used (1.2 ppm). Similar results have been reported by other groups. Gordon and Amdur (1980) and Gordon et al. (1984) found increased airway responsiveness to histamine after O_3 exposure to 0.8 or 1.2 ppm but not to lower O_3 concentrations. Although this finding could suggest that O_3 levels found in urban pollution do not cause airway hyperresponsiveness to histamine, we found a clear concentration-response relationship between O_3 exposure and airway reactivity to histamine, i.e. the higher the O_3 concentration, the higher the airway reactivity to histamine. In this context, it is likely that O_3 concentrations found in air pollution cause subtle changes in airways which could be important in those conditions such as asthma and chronic bronchitis, where enhanced airway responsiveness is already present.

Some studies using high O_3 concentrations (3 ppm) have demonstrated airway hyperresponsiveness to substance P both in in vivo and in vitro experiments (Yeadon et al., 1992; Murlas et al., 1992; Campos et al., 1992). Now we reported here that such hyperresponsiveness could be observed from 0.3 ppm, a concentration frequently found in the atmosphere of highly polluted cities such as Mexico City. The main proposed mechanism of this O_3-induced airway hyperresponsiveness to substance P is oxidative destruction of neutral endopeptidases located in airway epithelial cells, smooth muscle and other cells (Kummer and Fisher, 1991). Since this enzyme is responsible for substance P inactivation, damage of this enzyme would increase the effect of exogenous substance P.

It has been suggested that neurotransmitters such as substance P released during axon reflexes from the non-adrenergic non-cholinergic fibers are involved in the pathogenesis of asthma (Barnes, 1991). In this sense, pathological studies of airways from asthmatic patients have found either an increase in the number of substance P containing fibers (Ollerenshaw et al., 1991) or a decrease of immunoreactivity for such fibers, which could be interpreted as an enhanced release of mediators (Lilly et al., 1995). Thus, according to our results, exposure to high urban O_3 concentrations could lead to an enhanced effect of non-adrenergic non-cholinergic constrictor mediators in asthmatic patients. This could partially explain the epidemiological association between high levels of O_3 pollution and increased frequency of asthma attacks (Romieu et al., 1995). Nevertheless, tolerance has been described for some pulmonary function tests after repeated O_3 exposures (Folinsbee, 1992). Thus, chronic exposure to these or lower O_3 concentrations, as occurs in polluted cities, should be explored in order to determine if tolerance also develops for airway hyperresponsiveness to substance P.

Finally, due to the inverse relationship between respiratory frequency and lung ozone-absorption (Postlethwait et al., 1994), it could be speculated that noxious effect of O_3 would be higher in humans (± 12 breaths/min) than in guinea pigs (± 130 breaths/min). This agrees with the fact that the minimum O_3 concentration required to induce airway hyperresponsivess is lower in humans (0.08 ppm, McDonnell et al., 1991) than in animals (0.5 ppm, Abraham et al., 1980). Therefore, although we found that 0.3 ppm was the threshold for our guinea pig model of ozone-induced airway hyperresponsiveness, this threshold could be even lower for humans.

In conclusion, these findings suggest that excitatory non-adrenergic non-cholinergic responses could be affected by urban air pollution, and that substance P seems to be a better pharmacological tool than histamine and acetylcholine to demonstrate O_3-induced airway hyperresponsiveness at low concentrations which could be found in highly-polluted cities.

Acknowledgements

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