Ventilatory responses to ozone are reduced in immature rats

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Shore, S. A., J. H. Abraham, I. N. Schwartzman, G. G. Krishna Murthy, and J. D. Laporte. Ventilatory responses to ozone are reduced in immature rats. J. Appl. Physiol. 88: 2023–2030, 2000.—During ozone (O3) exposure, adult rats decrease their minute ventilation (Ve). To determine whether such changes are also observed in immature animals, Sprague-Dawley rats, aged 2, 4, 6, 8, or 12 wk, were exposed to O3 (2 ppm) in nose-only-exposure plethysmographs. Baseline Ve normalized for body weight decreased with age from 2.1 ± 0.1 ml·min⁻¹·g⁻¹ in 2-wk-old rats to 0.72 ± 0.03 ml·min⁻¹·g⁻¹ in 12-wk-old rats, consistent with the higher metabolic rates of younger animals. In adult (8- and 12-wk-old) rats, O3 caused 40–50% decreases in Ve that occurred primarily as the result of a decrease in tidal volume. In 6-wk-old rats, O3-induced changes in Ve were significantly less, and in 2- and 4-wk-old rats, no significant changes in Ve were observed during O3 exposure. The increased baseline Ve and the smaller decrements in Ve induced by O3 in the immature rats imply that their delivered dose of O3 is much higher than in adult rats. To determine whether these differences in O3 dose influence the extent of injury, we measured bronchoalveolar lavage protein concentrations. The magnitude of the changes in bronchoalveolar lavage induced by O3 was significantly greater in 2- than in 8-wk-old rats (267 ± 47 vs. 165 ± 22% respectively, P < 0.05). O3 exposure also caused a significant increase in PGE2 in 2-wk-old but not in adult rats. The results indicate that the ventilatory response to O3 is absent in 2-wk-old rats and that lack of this response, in conjunction with a greater specific ventilation, leads to greater lung injury.

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the animals of various ages. The tube was fitted with a silicone rubber gasket designed to fit snugly around the animal's neck and seal the head from the rest of the body. Once the animal was in the tube, a large piston was moved into place behind the animal. The piston served to prevent the animal from moving and to seal the body chamber from the outside air. Air displaced at the body surface as the animal breathed, passed across a pneumotachograph (8-mm diameter fitted with a screen filter) attached to a differential pressure transducer (model 163PC1D75, Omega Engineering, CT). The resulting flow signal was analyzed by a computer program (BUXCO, Troy, NY) that computed $V_{\dot{E}}, V_T$, breathing frequency, inspiratory (Ti), and expiratory time (Te) on a breath-by-breath basis and reported the average of each of these values every minute. The cranial end of the tube was inserted through a port in the Plexiglas door of a stainless steel chamber (−145 liters in volume). The animals were first exposed to filtered air for 45 min; the first 25 min of this period were used to adapt the animals to the plethysmographs and the last 20 min were used to obtain baseline values. The animals then either continued to be exposed to filtered air, or the air in the exposure chamber was switched to 2 ppm O3. Two ppm O3 exposure to air or O3 proceeded for an additional 3 h. For each ventilatory parameter, mean values over the 20 min immediately before O3 exposure were determined for each animal, and data are reported as percent changes from those values. In each animal, 5-min averages around the time point 10 min after initiation of O3, and at every 20 min thereafter, were computed. The effect of O3 exposure and age on these parameters was assessed by repeated-measures ANOVA.

O3 exposure O3 was generated by passing dry 100% oxygen through ultraviolet light and mixed with filtered room air in the chamber. Chamber atmosphere was drawn continuously via a sampling port, and O3 concentration was measured by an O3 chemiluminescent analyzer (model 49, Thermoelectron Instruments, Hopkinton, MA), which was calibrated by an ultraviolet photometric O3 calibrator (model 49PS, Thermoelectron Instruments). BAL. In a separate cohort of rats exposed in the same manner as described in Monitoring ventilation, a BAL was performed either immediately after or 4 h after the cessation of exposure to O3 or air. For these procedures, rats were killed by an overdose of halothane. The trachea was cannulated with a tubing adaptor, and phosphate buffered saline (0.035 ml/g) was gently inserted into the trachea and then withdrawn. This procedure was repeated two times. The lavage fluids were centrifuged at 400 g at 4°C for 10 min. The supernatant was frozen and subsequently analyzed for protein concentration by using the Bradford technique. An aliquot of the supernatant was recentrifuged at 60,000 g at 4°C for 30 min and subsequently analyzed for PGE2 by using an enzyme immunoassay kit (Caymen Chemical, Ann Arbor, MI). The antibody to PGE2 had <1% cross-reactivity to 6-keto-PGF1α, and <0.01% to thromboxane B2 and other prostaglandins according to the manufacturer’s specifications. The pelleted cells were resuspended in saline, and the number and type of cells were determined as follows. A well-mixed sample from each lavage return was cytocentrifuged onto microscope slides (Cytospin 2, Shandon Southern Instruments, Sewickley, PA), air dried, and stained with Wright-Giemsa stain (VWB Stat Stain, Brisbane, CA). From these slides, a differential count of 600 cells was performed. The total number of cells was determined by counting on a hemocytometer.

Statistics. All statistical analyses were carried out by using Intercooled STATA version 6.0 (College Station, TX). ANOVA performed with Bonferroni post hoc analysis was used to compare different rat age groups. In assessing ventilatory pattern changes within groups over time during air and O3 exposure, repeated-measures ANOVA was conducted to correct for the lack of independence of correlated observations. Univariate linear regression was used to assess the association between the animal age and the percent increase in BAL protein after O3 exposure. Age was coded by using 8-wk-old rats as a reference group and indicator variables for rats aged 2 and 4 wk.

RESULTS

Baseline ventilatory parameters. There was a marked difference, an almost 10-fold increase, in body weight between rats aged 2 and 12 wk (P < 0.001; Table 1). Baseline $V_E$ (i.e., $V_E$ measured before the onset of O3 exposure) also increased with age (P < 0.001), consistent with the increase in size of the older rats, but, when normalized for body weight, the immature (2- and 4-wk-old) rats were found to have a greater specific ventilation ($V_E/g$) than the older (6-, 8-, and 12-wk-old) rats (P < 0.002). The latter observation is consistent with the greater metabolic rate of younger animals. Age-related differences in $V_E$ were primarily the result of differences in $V_T$, which also increased with age (P < 0.001), whereas breathing frequency did not vary significantly across age groups, except in the 4-wk-old rats in which frequency was significantly higher than in all other age groups. Although there were differences in Ti and Te across certain age groups, these changes were not consistent with increasing age.

### Table 1. Age, body weight, and baseline ventilatory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2 (n = 19)</th>
<th>4 (n = 4)</th>
<th>6 (n = 13)</th>
<th>8 (n = 7)</th>
<th>12 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>34 ± 1</td>
<td>71 ± 10</td>
<td>134 ± 14</td>
<td>271 ± 15</td>
<td>312 ± 7</td>
</tr>
<tr>
<td>$V_E$, ml/min</td>
<td>73.5 ± 3.7</td>
<td>117.9 ± 9.6</td>
<td>106.7 ± 8.6</td>
<td>180.5 ± 13.9</td>
<td>223.6 ± 8.7</td>
</tr>
<tr>
<td>$V_E/60$-min⁻¹·g⁻¹</td>
<td>2.1 ± 0.08</td>
<td>1.8 ± 0.34</td>
<td>0.88 ± 0.09</td>
<td>0.68 ± 0.06</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>$V_T$, ml</td>
<td>0.49 ± 0.02</td>
<td>0.60 ± 0.04</td>
<td>0.80 ± 0.07</td>
<td>1.59 ± 0.11</td>
<td>1.74 ± 0.12</td>
</tr>
<tr>
<td>$V_E/g$, ml/g</td>
<td>0.0142 ± 0.0004</td>
<td>0.0089 ± 0.0012</td>
<td>0.0065 ± 0.0006</td>
<td>0.0056 ± 0.0005</td>
<td>0.0056 ± 0.0004</td>
</tr>
<tr>
<td>f, breath/min</td>
<td>149 ± 4</td>
<td>200 ± 14</td>
<td>137 ± 6</td>
<td>132 ± 15</td>
<td>132 ± 14</td>
</tr>
<tr>
<td>Ti, s</td>
<td>0.190 ± 0.007</td>
<td>0.141 ± 0.007</td>
<td>0.184 ± 0.017</td>
<td>0.286 ± 0.03</td>
<td>0.176 ± 0.016</td>
</tr>
<tr>
<td>Te, s</td>
<td>0.226 ± 0.010</td>
<td>0.180 ± 0.028</td>
<td>0.280 ± 0.014</td>
<td>0.248 ± 0.033</td>
<td>0.305 ± 0.026</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals in each group including both air- and ozone-exposed rats. $V_E$, minute ventilation; $V_T$, tidal volume; f, breathing frequency; Ti and Te, inspiratory and expiratory time, respectively; $V_E/g$ and $V_T/g$, specific $V_E$ and specific $V_T$, respectively.
Ventilatory responses to O₃. As previously described (32), exposure to O₃ caused a marked decrease in Vₑ in 12-wk-old rats (Fig. 1A). The decrease in Vₑ was significant (P < 0.001) within 50 min of the onset of O₃ exposure and remained so throughout the remainder of the O₃-exposure period. After 3 h of exposure to O₃, Vₑ averaged only 50% of baseline values. The decrease in Vₑ occurred primarily as the result of a decrease in VT (Fig. 1B), which occurred within 50 min of exposure (P < 0.001) and remained depressed throughout the rest of the exposure period. Changes in frequency were not statistically significant except toward the end of the exposure period, between 120 and 180 min (P < 0.05; Fig. 1C). Nevertheless, there were changes in the timing of ventilation such that Ti decreased (Fig. 1D) and Te increased (Fig. 1E). The decrease in Ti and the increase in Te were significant within 70 min of the initiation of O₃ exposure (P < 0.01 and P < 0.02, respectively). With further examination, the increase in Te was found to result primarily from an increase in end-expiratory pause (EEP; Fig. 1F), which was significant (P < 0.02) within 70 min of the onset of O₃ exposure. Indeed, in some animals, 1- to 2-s apneas were occasionally observed during O₃ exposure. Similar results were obtained in 8-wk-old rats. Because there were no statistically significant differences in the magnitude of O₃-induced changes in the various parameters among these two groups, the data from these 8- and 12-wk-old rats were combined and are described below as “adult” animals. Results from the other age groups were compared with the combined data from these adult animals. There was no statistically significant change in any ventilatory parameter during exposure to filtered air in 12-wk-old rats (Fig. 2).

Animal age had a marked effect on O₃-induced changes in Vₑ. Whereas the adult animals had prominent decreases in Vₑ over time with O₃ exposure, the responses in the 6-wk-old rats were less marked although still significant (P < 0.05). In the 2- and 4-wk-old animals, no significant effect of O₃ on Vₑ was observed at any time point after the initiation of O₃ exposure (Fig. 1A). The effect of O₃ on Vₑ was significantly greater in adult rats compared with each of the 2-, 4-, and 6-wk-old rats (P < 0.001 in each case). Similar results were obtained with VT, Ti, Te, and EEP. In each case, the O₃-induced response increased with animal age, being small or
near absent in the 2- and 4-wk-old rats, intermediate in the 6-wk-old rats, and marked in the 8- and 12-wk-old rats (Fig. 1, B, D, E, and F). For each of these ventilatory parameters, the effect of O$_3$ was significantly greater in adult rats compared with each of the 2-, 4-, and 6-wk-old groups ($P < 0.001$ in each case). Only breathing frequency, which showed little or no change in response to O$_3$ in any age group, failed to demonstrate statistically significant age-related differences. As in the adult animals, exposure to filtered air had no effect on ventilatory pattern in the younger animals (Fig. 2).

Effect of O$_3$ exposure on BAL protein, PGE$_2$, and neutrophils. The increased specific ventilation and the absence of a decrease in specific ventilation on O$_3$ exposure in the immature rats indicated that the effective inhaled dose of O$_3$ was greater in these animals. To determine whether this increase in dose resulted in enhanced injury or inflammation in the immature rats, we measured protein, PGE$_2$, and neutrophils in BAL fluid in 2-, 4-, and 8-wk-old rats immediately after and 4 h after exposure to O$_3$ (2 ppm for 3 h) and compared the results with animals exposed to filtered air. We used 8-wk-old rats to represent the adult response because the ventilatory response to O$_3$ was fully developed by this age (Fig. 1). We compared these rats with 2- and 4-wk-old rats because the difference in O$_3$ dose compared with the adults was greatest in these youngest animals.

Because there were age-related differences in BAL protein in the air-exposed animals (39 ± 5, 203 ± 51, and 180 ± 41 µg/ml in 2-, 4-, and 8-wk-old rats, respectively; $n = 5–6$ in each group), in O$_3$-exposed rats, differences in BAL protein responses to O$_3$ across age groups were computed as a percentage of the mean age-appropriate values from air-exposed rats. Compared with air exposure, in all three age groups, there was no difference in BAL protein obtained immediately after cessation of O$_3$ exposure, but there was a statistically significant increase in BAL protein 4 h after cessation of O$_3$ exposure ($P < 0.005$, $P < 0.01$, and $P < 0.05$ for 2-, 4-, and 8-wk-old rats, respectively). Four hours after O$_3$ exposure, BAL protein expressed as a percentage of air-exposed controls was significantly higher in 2-wk-old rats compared with 8-wk-old rats (267 ± 47 vs. 165 ± 22%; $P < 0.05$; Fig. 3). BAL protein expressed as percentage of values from air-exposed controls was also higher in 4- than in 8-wk-old rats, but this difference was not statistically significant (199 ± 21 vs. 165 ± 22%; $P = 0.5$).

There was also an effect of age on O$_3$-induced changes in the concentration of PGE$_2$ in BAL fluid. In 2-wk-old
rats, O$_3$ exposure caused a significant increase in PGE$_2$ in BAL fluid harvested from animals immediately after the cessation of O$_3$ exposure compared with air-exposed controls ($P < 0.01$; Fig. 4). Four hours after cessation of exposure, BAL PGE$_2$ had returned to air-exposed levels. This time course, in which PGE$_2$ peaked early after O$_3$ exposure and then declined, is consistent with that previously reported by Gunnison et al. (10, 11). In contrast, in both 4- and 8-wk-old rats, there was no significant effect of O$_3$ exposure on PGE$_2$ concentrations, whether BAL was performed immediately or 4 h after the cessation of O$_3$ exposure.

The total number of cells recovered from BAL fluid in air-exposed rats increased substantially with age from $8.8 \pm 1.5 \times 10^4$ cells in 2-wk-old rats to $26 \pm 3.2$ and $44.7 \pm 5.9 \times 10^4$ cells in 4- and 8-wk-old rats. Therefore, to compare O$_3$-induced changes in cell differentials across age groups, we expressed the number of each cell type as a percentage of the total cells in the BAL fluid of each animal (Fig. 5). In 8-wk-old rats, macrophages were the predominant cell type in BAL from air-exposed animals, followed by sloughed epithelial cells. Neutrophils accounted for only $\sim$1% of the total cells. After O$_3$ exposure, there was a marked increase in the number of neutrophils in the BAL fluid. There was a slight increase in epithelial cells, but it did not reach statistical significance. Lymphocytes and eosinophils represented $<1\%$ of cells during either air or O$_3$ exposure. Similar results were obtained in 4-wk-old rats, and the magnitude of the increase in the percentage of neutrophils after O$_3$ exposure was virtually identical to that in the 8-wk-old rats. In contrast, in 2-wk-old rats, there was no increase in the percentage of neutrophils in BAL fluid after O$_3$ exposure, although epithelial cells and protein concentration (Fig. 3) did increase significantly, indicating that there was airway injury.

**DISCUSSION**

Our results indicate that specific ventilation is greater in immature than adult rats (Table 1), consistent with a greater metabolic rate in the younger animals (22). Our results also indicate that O$_3$ exposure causes a marked, $\sim$40–50% decrease in V$_E$ in adult (8- and 12-wk-old) rats but that V$_E$ decreases minimally, if at all, with O$_3$ exposure in less mature rats (Fig. 1). Because the inhaled dose of O$_3$ is the product of O$_3$ concentration, exposure time, and V$_E$ (3, 21, 38), the net effect of the greater metabolic rate and the failure to decrease V$_E$ with O$_3$ in 2- and 4-wk-old rats is such that, in these rats, the inhaled dose of O$_3$, relative to lung size, is about five to six times greater than in adult rats. Our results confirm previous reports of decreases in V$_E$ during O$_3$ exposure in adult rats (1, 19, 32). The decrease in V$_E$ appears to be the result of a corresponding decrease in metabolic rate, because oxygen consumption and heart rate decrease (13, 19) but arterial PCO$_2$ is relatively unchanged with O$_3$ (34). It is likely that the decrease in metabolic rate arises, at least in part, from a regulated decrease in core body temperature, because
the latter decreases by as much as 2–3°C during O₃ exposure in adult rats (13, 19, 37). Similar to the time course of O₃-induced changes in Vₑ (Fig. 1), the decreases in temperature occur slowly after the initiation of O₃ exposure and persist for some time after the cessation of exposure before gradually returning to preexposure levels (13). This time course suggests that the formation and release of chemical mediators may be necessary to evoke these changes. One candidate for such factors is tumor necrosis factor-α (TNF-α), because its expression in alveolar macrophages increases after O₃ exposure (23) and it is capable of reducing core body temperature (16). Alternatively, it may be that the decrease in core body temperature is the consequence, not the cause, of the reduced metabolic rate and that the latter is driven by conscious and behavioral reductions in activity. Activity has been reported to decrease in rats acutely exposed to O₃ (37). Numerous afferents arising from the nose and upper and pulmonary airways are stimulated by O₃ (1, 6, 25, 26) and could constitute the sensory arm of the neural pathways that drive these changes in activity. Changes in the activity of these afferents may also contribute to the changes in the timing of ventilation observed in adult animals.

To our knowledge, the only published report of age-related differences in the ventilatory response to O₃ is one by Arito et al. (1). They compared young adult (4- to 6-mo-old) vs. aged (20- to 22-mo-old) rats and found no substantive differences in the magnitude of changes in Vₑ evoked by O₃, an observation consistent with that of Vincent et al. (36), who found no difference in the O₃ dose across the same ages of rats by using ¹⁸O₃ uptake as the index of dose. The data reported here are the first to look at ventilatory responses to O₃ in younger animals. Our results indicate that the decrease in Vₑ induced by O₃ is virtually absent in 2- and 4-wk-old rats, weak but present in 6-wk-old rats, and fully developed by 8 wk of age. We do not know the mechanistic basis for these age-related differences. If O₂-induced decreases in core body temperature and metabolic rate are caused by the elaboration of cryogenic factors such as TNF-α, then it may be that the production of such factors is suppressed in immature animals. There are reports of age-related increases in TNF-α production in rats and mice (27, 31). However, alveolar macrophages from young (6- to 7-wk-old) mice produce more TNF-α in response to stimulation with interferon-γ and lipopolysaccharide than do macrophages from older (26-wk-old) mice (12). It is also possible that the younger animals, which still lack full coats of fur, are more dependent on activity to maintain their core body temperature than are adult rats. If so, in immature rats, the need to maintain a high metabolic rate may override any signals arising from O₃ exposure that would tend to decrease activity, metabolic rate, and, consequently, Vₑ. Alternatively, it may be that the neural pathways driving O₂-induced changes in activity, like much of the central nervous system, are not completely developed in the younger animals.

Regardless of the mechanisms by which age-related differences in the ventilatory response to O₃ occur, it is clear that the consequences of these differences and of the elevated specific ventilation in young animals are such that the immature rats have an inhaled dose of O₃ that, relative to their size, is five to six times that observed in the young adult rats. To determine whether these differences in O₂ dose result in differences in lung injury or inflammation, we performed BAL after air or O₃ exposure in rats aged 2, 4, or 8 wk and measured protein and PGE₂ concentrations and cell differentials in the BAL fluid. In adult rats, O₃ at this concentration typically results in both an increase in BAL protein and an increase in the number of neutrophils in BAL fluid (32), and both have been used as standard indexes of O₃-induced injury. We found that BAL protein increased with O₃ exposure in all three age groups. The time course of these changes in BAL protein, in which changes were observed 4 hr after, but not immediately after, cessation of O₃ exposure, is consistent with other reports (33). As shown in Fig. 3, O₃-induced BAL protein extravasation decreased with increasing age, and significant differences were observed between the 2- and 8-wk-old mice. BAL PGE₂ also increased in 2- but not in 8-wk-old rats, although there was substantial variability in this response (Fig. 4). These results are consistent with greater O₃-induced lung injury caused by the greater dose of O₃ delivered to these younger animals.

Both 4- and 8-wk-old rats responded to O₃ with an increase in neutrophils, but there was no difference in the magnitude of this increase between these two age groups. Two-week-old rats failed to demonstrate any neutrophils in BAL fluid after O₃ (Fig. 4), despite increases in epithelial cell sloughing, PGE₂, and BAL protein, indicating that there was indeed airway injury and of a magnitude greater than that induced in the other age groups. We do not know why O₃-induced injury fails to induce neutrophil influx in the 2-wk-old rats. However, deficiencies in neonatal neutrophil emigration in response to other stimuli have been noted in very young rats, rabbits, and humans (9, 17, 18, 24). For example, Martin et al. (18) reported that there was virtually no increase in BAL neutrophils in response to intratracheal instillation of lipopolysaccharide in rats from newborn up to 2 wk of age, whereas adults had a robust response. It is unlikely that the absence of neutrophil emigration in these O₃-exposed 2-wk-old rats is the result of a diminished pool of circulating neutrophils, because blood neutrophils are actually present in greater numbers in these younger animals (18). Instead, reduced adhesion molecule expression and reduced chemotactic factor generation have been postulated to contribute to these deficiencies (9, 17, 24). Because of these deficiencies, BAL neutrophils are not likely to be an accurate index of O₃-induced lung injury in this youngest group of rats. Because neutrophils contribute to injury induced by some stimuli that induce their migration, it is also possible that the deficient neutrophil emigration observed in 2-wk-old rats results in a lesser degree of injury than might otherwise have been observed.
In contrast to very young rats, 4-wk-old rats respond to intratracheal instillation of endotoxin with a robust increase in BAL neutrophils, comparable to that observed in adult animals (18), suggesting that any deficiency in neutrophil migration has been resolved by this age. Hence, we believe that BAL neutrophilia is probably a valid index of O3-induced lung injury in the 4-wk-old rats. In this respect, it is interesting that the 4-wk-old rats had an increase in BAL neutrophils no greater than that observed in the adult rats (Fig. 5), despite an approximate fourfold greater inhaled dose of ozone. Similarly there were no differences in BAL PGE2 and only minor differences in BAL protein between 4- and 8-wk-old rats. Taken together, the results suggest that these younger rats may, in fact, be less sensitive to O3 than adults. Similarly, young rats are less sensitive to hyperoxia (5, 14), probably because of an increased ability to augment antioxidant defenses on exposure to oxidants (14, 39). A greater inhaled dose, but a reduced sensitivity to O3, may explain why some investigators report greater responses to O3 in young rats, whereas others report reduced responses (see below).

Relatively few studies have considered age as a factor in any aspect of the response to O3, and results vary depending on the species used. In mice, the results suggest that responses to O3 are more severe in younger animals (4, 30). O3 responses also decrease with increasing age in humans (21, 21), although only adults >18 yr of age have been examined. In rats, the results are less consistent and appear to depend on the O3 concentration, the exposure time, the age range, and the outcome indicator examined. Stiles and Tyler (29) reported that lungs of young adult rats (2 mo of age) had more extensive centriacinar lesions than did lungs of middle-aged rats (444 days old) after exposure to 0.35 and 0.8 ppm ozone for 72 h. Similarly, Elsayad et al. (7) reported that neonatal rats died after exposure to 0.8 ppm O3 for 3 days, whereas juvenile and adult rats did not. In contrast, Mustafa et al. (22) reported that mortality was greater in 12-wk-old rats than in 3- to 4-wk-old rats exposed to 4 ppm for 8 h. Barry et al. (2) did not find any difference in epithelial damage of 1-day-old or 6-wk-old rats exposed to 0.25 ppm for 6 wk, whereas Stephens et al. (28) reported that the terminal airways of neonatal rats between birth and weaning are very resistant to the cytotoxic effects of O3 (0.85 ppm for up to 72 h). The mechanistic basis for airway injury induced by such chronic O3 exposures is likely to be different from that induced by the acute (3 h) O3 exposures reported here. Vincent et al. (36) examined the age dependence of the inflammatory response to acute O3 exposure and found that there was no effect of age on either protein extravasation or neutrophil influx in response to O3 but there was increased release of interleukin-6 in older animals. However, they studied aging only in adult to senescent animals and not in neonates. Only Gunnison et al. (10, 11) have reported age-related effects on responses to such acute exposures in very young rats. Consistent with our results (Fig. 3), they observed that neonatal rats have greater responses to O3 than do juvenile or adult rats when PGE2 release into BAL fluid immediately after cessation of exposure is used as the outcome indicator. These greater responses are likely the result of a greater inhaled dose of O3 rather than of any increased sensitivity to O3. They also measured neutrophils and BAL protein but examined a time point well before substantive responses to O3 occur, which precluded them from drawing conclusions about those aspects of the O3 response.

In summary, our results indicate that immature rats have a higher Vc, normalized for body weight, than adult rats and that the decrease in ventilation observed on exposure of adult rats to O3 is not observed in immature rats. These changes in ventilation result in a marked increase in the inhaled dose of O3. In the youngest (2-wk-old) rats, this increase in dose results in greater lung injury as manifest by relatively greater increases in BAL protein and PGE2. However, BAL protein, PGE2, and neutrophils are not substantially different in 4-wk-old compared with adult rats, despite an approximate fourfold difference in O3 dose, suggesting that the immature rats may be less sensitive to the effects of O3.

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