INTRODUCTION

There are reasons to believe that the hypoxic ventilatory response decreases with advancing age. In humans, lung volumes, alveolar structures, and the amount of alveolar air all decrease in older age (1). Chest wall mechanics worsens and the respiratory muscle pump weakens (2-4). Moreover, there are degenerative age-related alterations in the carotid body (5, 6), which generates the hypoxic ventilatory response, and the carotid body sensory input to, and its elaboration in, the brainstem respiratory areas are expected to be diminished with age (7).

Despite the intuit that the above mentioned age-related alterations in the respiratory system should brought about a decrease in the hypoxic ventilatory response, the evidence is none all clear. Human studies, all cross sectional studying population samples of various age in the state of wakefulness, demonstrate a decrease (8, 9), no change (10, 11), or even an increase (12) in the hypoxic ventilatory response in older age; the latter being quite apparent in some reports (13).

The animal studies on the subject are sparse and their results are just as variable. Schlenker and Goldman (14) have shown that the slope of minute ventilation (\( V_{\text{E}} \)) on \( \text{PaO}_2 \), which describes hypoxic sensitivity of the respiratory system, increases in 24 months old, particularly female, conscious rats. In anesthetized rats, Fukuda (15) has found that the hypoxic \( V_{\text{E}} \), but not hypoxic sensitivity, is gradually attenuated with advancing age. However, the attenuation was lost when \( V_{\text{E}} \) was normalized to basal metabolic rate, which decreases with age.

The interpretation of alterations in hypoxic reactivity with advancing age is hampered by the paucity of pertinent studies, differential experimental conditions employed, and the lack of longitudinal studies in which the same animal would be tested to hypoxia with the passing time. In the current study we set out to examine alterations in hypoxic reactivity in senescent conscious rats. We addressed this issue by comparing the hypoxic ventilatory response in three age-groups of conscious rats: 3, 12, and 24 months old animals.

MATERIALS AND METHODS

Animals

The experiments were performed in accordance with bioethical requirements as per the Polish Animal Protection Bill of August 21, 1997 and the study protocol was approved by a local Ethics Committee.

A total of 22 male Wistar rats were used for the study. The animals were studied in the state of wakefulness throughout the
whole experimental protocol. There were two unrelated groups of rats. The rats of the first group (n=8) were studied once at the age of 3 months. The rats of the second group (n=14) were different and were studied twice: at the age of 12 and 24 months. At the time of the experiment the rats’ age could vary by ±2 weeks from the above mentioned group limits. The rats of the second group were kept continuously in the Animal House during the one-year study period. Therefore, this part of the study, between the rat middle age of 1 year and the senescent age of 2 years, had a longitudinal character. The rats were housed in groups of 3-4 in stainless steel mesh cages up to the age of 1 year and singly thereafter. All rats were kept under standard conditions on an artificial 12 h light-dark cycle, with the light on at 8 a.m., temperature of 21±2°C, and humidity of 50-60%. The animals had free access to rodent chow and tap water. All rats of all age-groups were subjected to the same experimental procedure.

Measurement of ventilation

Ventilatory measurements were made in a whole body rodent single-chamber plethysmograph (model PLY3223, Buxco Electronics, Wilmington, NC). The system consisted of a Plexiglas chamber equipped with two pneumotachographs. The chamber received a bias flow supply of 1.5 l/min fresh air in-between the hypoxic tests, necessary to remove CO2 build-up, via a flow pump reservoir system (PLY1020, Buxco Electronics). Pressure difference between the experimental and reference chambers was measured with a differential pressure transducer. The pressure signal was amplified (MAX1320 preamplifiers and interface; Buxco Electronics), and integrated by data analysis software (BioSystem XA for Windows SFT3410 v. 2.9, Buxco Electronics). The system was calibrated before each experiment with 10 ml of ambient air introduced into the chamber. Tidal volume (Vt), respiratory frequency (f), and minute ventilation (Vl ml/min, BTPS) were computed in the breath-by-breath routine throughout the experimental procedure and were stored in computer memory for off-line analysis. A 30-s average was taken for each variable during the baseline recording just before the commencement of hypoxic exposure. Thereafter, 10-s averages were taken before the half-minute time points during the course of hypoxic exposure.

Experimental procedure

After a 10-15 min acclimation of the rat to the chamber, breathing ambient air, the animal was exposed to two levels of decreasing inspired O2: 14 and 11% O2 in N2. The order of hypoxic exposures was chosen randomly. Hypoxic tests took 2 min, after which the chamber gas mixture was switched to air. Normoxic/hypoxic and vice versa gas switch took 40 s to achieve equilibrium of a given gas mixture inside the plethysmographic chamber, after which the time count of hypoxic exposure started. Hypoxic exposures were separated by a 15-min recovery interval in air. During the experiment the expired CO2 was allowed to run free. The rat body temperature was not controlled for during hypoxic exposures, as our control trials failed to show any appreciable temperature changes during short term hypoxic tests. Rectal temperature before consecutive hypoxic exposures was maintained at 37.0±0.5°C.

Data evaluation

All data are expressed as means±SE. Since the young rats constituted a separate group, even though the middle-aged and senescent rats were the same animals, the three age-groups were treated statistically as independent groups. There were substantial gains in animals' body mass with advancing age (see Table 1). Therefore, ventilatory variables were normalized to weight in kg. Statistical analysis of values representing the time profile of hypoxic responses was carried out using means calculated every 30 s.

Since the Shapiro-Wilk test rejected the null hypothesis that the data came from a normally distributed population, we used tests for not normally distributed data throughout the statistical elaboration. Significance of differences among the three age-groups of rats in weight, resting ventilatory variables, and at the sequential time points of the hypoxic response was estimated using the Kruskal-Wallis test followed, if required, by the Mann-Whitney U test. Significance of hypoxic Vl increases from baseline to peak at 0.5 mm and decreases from peak to depressant nadir at 2 min in each age-group was evaluated with the Friedman test followed by the Wilcoxon test for comparisons among these three time points. P<0.05 was deemed as indicative of significant differences.

RESULTS

Ventilation during baseline breathing

Baseline ventilatory variables during air breathing before the start of experimental runs are displayed in Table 1. The weight of rats increased with age. The increase was highest at the age of 12 months, amounting to 0.56±0.02 vs. 0.42±0.01 kg in the youngest age-group (P<0.03), and was sustained in the senescent rats, albeit with a declining tendency. Resting Vl amounted to 710.9±45.5 ml/min kg⁻¹ in the young rats, remained in the same range at 12 months of age, and significantly increased at 24 months of age to 937.7±72.8 ml/min kg⁻¹ (P<0.03). The increase in resting Ve was driven mainly by increasing f with advancing age. At the age of 24 months, the increase in Ve was strengthened by Vl that rebounded from a significant decline at 12 months (Table 1).

Hypoxic ventilatory response profiles

Profiles of the Ve responses to 14 and 11% hypoxia are presented in Fig. 1 (Panel A and B, respectively). All responses showed a characteristic stimulatory/inhibitory pattern, albeit the ventilatory fall-off was rather small during a short, 2-min hypoxic test duration. The peak increase in Ve, the point of major interest in the hypoxic response, was noted, on average, at 0.5 min from the completion of switching the inspiratory gas mixture to hypoxia. In the youngest rats, the mean peak Ve increases were from 726.7±30.6 to 833.2±57.8 ml/min kg⁻¹ and from 702±30.6 to 980.1±55.4 ml/min kg⁻¹ during 14 and 11% hypoxia, respectively. From there, the ventilatory fall-off started

Table 1. Ventilatory variables during baseline air breathing in the age-groups of rats.

<table>
<thead>
<tr>
<th>Age</th>
<th>3 mo (n=8)</th>
<th>12 mo (n=14)</th>
<th>24 mo (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>0.42±0.01</td>
<td>0.56±0.02</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>Vl (ml/min·kg⁻¹)</td>
<td>710.9±45.5</td>
<td>730.1±37.7</td>
<td>937.7±72.8</td>
</tr>
<tr>
<td>f (breaths·min⁻¹)</td>
<td>87.1±3.7</td>
<td>119.3±6.5</td>
<td>119.5±8.2</td>
</tr>
<tr>
<td>Ve (ml/kg)</td>
<td>8.4±3.0</td>
<td>6.9±0.4</td>
<td>8.0±0.4</td>
</tr>
</tbody>
</table>

Values are means±SE. There were significant differences among the three age-groups of rats; (•) 24 vs. 12 months old, (†) 24 vs. 3 months old, (#) 12 vs. 3 months old (Kruskal-Wallis test followed by Mann-Whitney U test, P<0.03).
down to 791.0±51.8 and 884.2±66.2 ml min⁻¹ kg⁻¹ at the respective concentrations of inspired oxygen, at 2 min of hypoxic exposure, the ending time point of the test. The peak $V_E$ increase and fall-off achieved significance ($P<0.02$) at the stronger 11% hypoxia. The magnitude of hypoxic $V_E$ responses of 12 months old rats failed to show an appreciable difference from those of the young ones (Fig. 1). Hypoxic $V_E$ responses of the senescent rats, on the other hand, were strikingly enhanced. The response profiles were shifted upward along the course of the test compared with those of 3 and 12 months old rats. In the senescent rats, the mean peak hypoxic $V_E$ increases were from 816.2±72.9 to 1099.1±105.6 ml min⁻¹ kg⁻¹ and from 853.5±77.8 to 1463.3±179.2 ml min⁻¹ kg⁻¹ during 14 and 11% hypoxia, respectively ($P<0.01$ for both). The ventilatory fall-off, starting from the peak $V_E$ increase, as opposed to the youngest rats, was less clear and tended to stabilize toward the test-end. This enhancement of the fall-off level caused that there were significant differences in $V_E$ representing the three age-groups of rats at the sequential time points (vertically distributed in Fig. 1) along the hypoxic response course, which became clearly accentuated at the stronger hypoxic stimulus.

The biphasic profile of hypoxic $V_E$ was predominantly reflected in $V_T$ changes (Fig. 2), with a smaller contribution of $f$. The latter became more pronounced during the stronger hypoxia, and in the older age-groups (Fig. 3). In general, $V_T$ decreased and $f$ increased at 12 and 24 months of age. These changes persisted during the course of both levels of hypoxia and may be best exemplified by the peak 0.5 min responses. A decrease in peak hypoxic $V_T$ was strongest at 12 months of age, with a rebound toward the 3-month level in the senescent rats (Fig. 2), whereas $f$ increased across the middle and senescent ages; the effect being stronger at the stronger hypoxic level (Fig. 3B). An increase in $f$ sustained the hypoxic $V_E$ in the 12 months old rats at the level of that present in the 3 months old rats, in the face of a substantial decrease in $V_T$, and it also collaborated with the bouncing back $V_T$ in enhancing hypoxic $V_E$ in the senescent rats.

*Fig. 1. Minute ventilation ($V_E$) changes during exposure to 14% $O_2$ (Panel A) and 11% $O_2$ (Panel B) in rats aged 3, 12, and 24 months. Lack of SE bars depicts SE not exceeding the symbol size. Significant differences among the three age-groups of rats at the sequential (vertical) time points of the hypoxic response are marked as follows: (*) 24 vs. 12 months old and (†) 24 vs. 3 months old rats ($P<0.05$ for Panel A and $P<0.01$ for Panel B).

*Fig. 2. Tidal volume ($V_T$) changes during exposure to 14% $O_2$ (Panel A) and 11% $O_2$ (Panel B) in rats aged 3, 12, and 24 months. Lack of SE bars depicts SE not exceeding the symbol size. Significant differences among the three age-groups of rats at the sequential (vertical) time points of the hypoxic response are marked as follows: (*) 24 vs. 12 months old, (†) 24 vs. 3 months old, (#) 12 vs. 3 months old ($P<0.02$ for Panel A and $P<0.05$ for Panel B).

*Fig. 3. Breathing frequency ($f$) changes during exposure to 14% $O_2$ (Panel A) and 11% $O_2$ (Panel B) in rats aged 3, 12, and 24 months. Lack of SE bars depicts SE not exceeding the symbol size. Significant differences among the three age-groups of rats at the sequential (vertical) time points of the hypoxic response are marked as follows: (*) 24 vs. 12 months old, (†) 24 vs. 3 months old, (#) 12 vs. 3 months old ($P<0.01$ for Panel A and $P<0.02$ for Panel B).
The hypoxic ventilatory gain may be assessed from the increase in peak hypoxic $V_T$ with increasing strength of the hypoxic stimulus. The $V_T$ increase achieved at the stronger 11% hypoxia, in each age-group is shown in Fig. 4. Statistical significance of changes in the peak hypoxic $V_T$ is already given in Fig. 1 (Panels A and B – 0.5 min points). The inset in Fig. 4 depicts the mean increments in peak $V_T$, calculated from individual differences between the peak $V_T$ achieved in response to 11 and 14% hypoxia in each age-group. The increment in peak $V_T$ amounted to $364.1 \pm 95.8 \text{ ml min}^{-1} \text{kg}^{-1}$ in 24 months old rats and was significantly greater than that in 12 or 3 months old rats ($96.1 \pm 36.2$ and $146.9 \pm 28.8 \text{ ml min}^{-1} \text{kg}^{-1}$, respectively; $P<0.02$); the difference in peaks between the latter two was inappreciable. Therefore, at a given level of hypoxia not only was the peak $V_T$ higher in the senescent rats, but also the hypoxic ventilatory gain was augmented.

DISCUSSION

In this study we set out to examine changes in the hypoxic ventilatory response developing with progressing age of rats, from young adulthood to senescence. The study demonstrates that the hypoxic ventilatory response in 12 months old rats was akin to that in the youngest 3 months old rats studied, whereas the response substantially increased in senescent rats. The increase in the response was noted in the prospective part of the study, as 12 months and 24 months old rats were the same animals studied one year apart.

The increased hypoxic ventilatory response in old age was a rather unexpected finding, since there is a general consensus that the respiratory system lacks efficiency in old age. The majority of human studies point to the possibility of age-related dampening of ventilatory responsiveness (8, 9). The carotid bodies, which generate the hypoxic ventilatory response, age morphologically (5, 6) and the functionality of oxygen sensitive mechanisms in the carotid body is age-dependent (16). Deficient antioxidant defenses and repetitive episodes of arterial blood desaturations, both often accompaniments of old age, may bear on decreased plasticity of chemosensory responses as well (17, 18).

The issue is, however, far from being settled. Comparison of the present results with the literature data is hampered by the scarcity of pertinent studies. In awake humans, apart from the studies showing an attenuation of the hypoxic ventilatory response (8, 9), there are reports pointing to increased responses with age (12, 13). Animal studies on the issue are even scarcer. Schlenker and Goldmann (19) have shown that with the progressing age from 3 to 12 months basal $V_T$, tidal volume, inspiratory flow rate, and hypoxic responsiveness all increase in magnitude in awake male rats. The opposite, decreases in basal ventilatory variables in one-year interval, from 12 to 24 months, were found in another study by the same authors, although the scatter of data was substantial and there were gender differences (14). The decreases were apparent in both genders, but they reached a clear significance only in male rats. Despite the decreases in basal $V_T$, hypoxic ventilatory responsiveness, assessed from the slope of $V_T$ on $P_{O_2}$, more than doubled in female rats, although its increase in male rats was less conspicuous due to a large variability of responses. Characteristically, $V_T$ contribution to the increased hypoxic responsiveness dominated in both genders (14).

Fukuda (15) has shown that the magnitude of the $V_T$ increase in response to hypoxia progressively declined with age up to 20 months in male rats. The decline was unaccompanied by a decrease in hypoxic sensitivity, even though the rats were anesthetized with halothane, an anesthetic known to dampen the carotid body-mediated sensory chemoreflex (20). Moreover, when ventilation was normalized to metabolic rate, the age-related attenuation was no longer there. The author ascribes age-changes in hypoxic $V_T$ to reduced $O_2$ consumption with age (15). In a recent study, Wenninger et al (21) have assessed the effects of age and gender on the hypoxic and hypercapnic ventilatory responses in young, middle-aged, and senescent awake rats. The authors used an experimental paradigm in which the hypoxic ventilatory response was assessed from a change in the ventilatory equivalent, i.e., $\Delta V_{E}$-to-oxygen consumption ratio, and only one level of the hypoxic stimulus was used. They found no significant age-related changes in either hypoxic or hypercapnic response in either gender, although senescent female rats had a higher hypoxic response than that in male rats. Interestingly, the ventilatory response to $CO_2$, which was decreased in 1-year old male rats, rebounded in >2-years old rats.

Our present results are in parts concordant with the literature data outlined above: a well conserved hypoxic ventilatory response in the aged rats, or an increase in hypoxic responsiveness from 12 to 24 months of age and an appreciable role of $V_T$ in the increase; and in parts discordant: an increase also in basal $V_T$ from 12 to 24 months or unchanged hypoxic responsiveness from 3 to 12 months. The reasons for the discordance are not readily apparent. They may have to do with methodological differences and a complex and as yet not well understood interaction between central and peripheral ventilatory mechanisms and age. However, having studied two levels of hypoxia we expand on the many of previous studies on the subject by showing that ventilatory gain actually increased in the aged rats.

The enhanced hypoxic ventilatory response in senescent rats indicates that the respiratory system has got ways to compensate...
for the possible untoward age-changes in the neural and muscular-skeletal structures. A few explanations for the compensation can be posited, involving both central and peripheral mechanisms. The hypoxic chemoreflex is generated in the paired sensory organ of the carotid body and is subject to the brain stem respiratory integrative mechanisms. The latter, in turn, are under cortical influence in wakefulness. Cortical input is integrated at the medullary level, as magnetic stimulation applied through the skull is capable of activating both on- and off-switch mechanisms of the breathing pattern (22). Cortical signal may also be directly relayed to the spinal respiratory motoneurons (23), as stimulation of the cortex evokes responses in respiratory motoneurons (24, 25).

Acute hypoxia causes ventilatory stress that is sensed and elaborated by cortical activity (26), and thus may modulate the respiratory signal running down to the diaphragm. On the peripheral side, vagally-mediated lung reflexes may play a greater modulatory role during increased chemoreflex drive (27).

Carotid body parenchyma exhibits substantial age-changes in senescence. These changes consist of chemoreceptor cell degeneration, which should lead to a functional fall-out of some of the chemoreceptor cells. Age-changed chemoreceptor cells abut, however, with the cells that have a normal morphologic look to them (5, 6). Therefore, a consistent impression arises that there is a developmental surplus of chemoreceptor cells in the carotid body. A fraction of the functional chemoreceptor cell could suffice to initiate the chemoreflex or, perhaps, the less the firing of carotid sensory discharges the stronger the amplification of it at the brain stem level to maintain the hyperventilatory response. That the carotid body remained still an active player shaping the senescent ventilatory responsiveness may be inferred from a notable contribution of the frequency of breathing to increased resting ventilation and hypoxic ventilatory responses. Frequency changes are a characteristic feature of carotid body mediation in rodents (28).

Cortical influences may be one of those factors that downgrade the explicit role of the carotid bodies in maintaining the hypoxic ventilatory response. In awake dogs with chronic carotid body denervation, some ventilatory response to acute hypoxia is still preserved (29). Likewise, in awake rats other than the carotid body peripheral and central mechanisms contribute to the hypoxic ventilatory response (30, 31). In the guinea pig, the carotid body does not seem to underlie the hypoxic ventilatory response at all (32). The role of such mechanisms in the aged rat is open to conjecture, but they could gain in importance in the face of chronic age-related morphologic carotid body insufficiency (5, 6).

Proliferation of connective tissue in carotid body parenchyma (5) is bound to induce chronic tissue hypoxia. Such hypoxia may reset basal activity of the organ to a higher level of excitation, from which it responds to hypoxia; thus achieving a higher level of excitation during hypoxia. That could be one another mechanism resulting in maintaining the hyperventilatory response. In relation to connective tissue outgrowth in senescence, enhanced ventilatory drive also could arise from concomitantly higher tissue, or otherwise, CO₂ partial pressures at any level of inspiratory O₂ fraction due to worsened lung gas exchange. The CO₂-related drive seems rather dubious in view of decreased CO₂ production (14), apparent occurrence of cellular hypoxia at a relatively higher end-tidal PO₂ (15), and an overall decreased metabolic rate with advancing age (33). The resolution of this issue would require direct metabolic rate measurements; the lack of which is one of the limitations of the present study. It is, however, hardly feasible to measure the metabolic rate during transient responses with the currently available techniques.

The study has other limitations. The hypoxic stimuli we used were fairly mild, which makes it unlikely that the respiratory system was strained to its maximum. Had it been strained more profoundly, a reduction in hypoxic responses in the aged rats could have come into sight. However, the mild hypoxic stimulation was used purposefully in this study. Senescent rats are vulnerable and erratic in their responses to stressful stimuli, in particular to repeat stimulation, as was the case in the present study. A milder strength of hypoxic stimulation allowed to conclude the tests as uneventfully as possible, which lessened the variability of results and the appearance of untoward effects such as agitation and movement artifacts, and thus allowed, we believe, for a more meaningful comparisons among the age-groups. Finally, it should be mentioned that the CO₂ was allowed to run free throughout the hypoxic tests. Hypocapnia, bound to develop during hypoxic hyperventilation, would, however, mitigate the increase in hypoxic responsiveness.

In conclusion, we found that the hypoxic ventilatory response and hypoxic ventilatory gain are not curtailed in senescent awake rats. The corollary is that age alone is not necessarily linked to deficient lung ventilation, and, consequently, to decreased O₂ supply to tissues in response to ventilatory stress. The respiratory system is able to compensate for any age-related handicaps to maintain ventilatory responsiveness.

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