Potentiation of hypoxic ventilatory response by hyperoxia in the conscious rat: putative role of nitric oxide

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Gozal, David. Potentiation of hypoxic ventilatory response by hyperoxia in the conscious rat: putative role of nitric oxide. J. Appl. Physiol. 85(1): 129–132, 1998.—In humans, the hypoxic ventilatory response (HVR) is augmented when preceded by a short hyperoxic exposure (Y. Honda, H. Tani, A. Masuda, T. Kobayashi, T. Nishino, H. Kimura, S. Masuyama, and T. Kuriyama, J. Appl. Physiol. 81: 1627–1632, 1996). To examine whether neuronal nitric oxide synthase (nNOS) is involved in such hyperoxia-induced HVR potentiation, 17 male Sprague-Dawley adult rats underwent hypoxic challenges (10% O2-5% CO2-balance N2) preceded either by 10 min of room air (–O2) or of 100% O2 (+O2). At least 48 h later, similar challenges were performed after the animals received the selective nNOS inhibitor 7-nitroindazole (25 mg/kg ip). In –O2 runs, minute ventilation (Ve) increased from 121.3 ± 20.5 (SD) ml/min in room air to 191.7 ± 23.8 ml/min in hypoxia (P < 0.01). After +O2, Ve increased from 114.1 ± 19.8 ml/min in room air to 218.4 ± 47.0 ml/min in hypoxia (+O2 vs. –O2: P < 0.005, ANOVA). After 7-nitroindazole administration, HVR was not affected in the –O2 treatment group with Ve increasing from 113.7 ± 17.8 ml/min in room air to 185.8 ± 35.0 ml/min in hypoxia (P < 0.01). However, HVR potentiation in +O2-exposed animals was abolished (111.8 ± 18.0 ml/min in room air to 184.1 ± 35.6 ml/min in hypoxia; +O2 vs. –O2: P not significant). We conclude that in the conscious rat nNOS activation mediates essential components of the HVR potentiation elicited by a previous short hyperoxic exposure.

hypoxia; hyperoxia; nitric oxide; respiratory control

METHODS

Animals. The experimental protocols were approved by the Institutional Animal Use and Care Committee. Survival experiments were performed on 17 male Sprague-Dawley young adult rats (175–200 g). In a preliminary stage, anesthesia was induced by pentobarbital sodium (Nembutal, 50 mg/kg ip). Rectal temperature was monitored by a Harvard thermal probe to maintain core temperature at 37–37.5°C by a servo-controlled heating pad. A 1-cm incision of the groin was performed, and indwelling polyethylene catheters (PE-50, 0.56-mm ID, 0.88-mm OD) were surgically placed in the femoral artery and vein. Catheters were advanced −5−7 cm to reach the abdominal aorta and inferior vena cava, then were secured, tunneled subcutaneously, exteriorized in the dorsal aspect of the neck, flushed with a heparin-containing solution (1,000 U/ml saline), sealed with heat, and stored in a cap sutured to the skin. Animals were then allowed to recover for at least 72 h, as demonstrated by return to normal feeding and sleep-waking schedules. Animals were provided with water and rat chow ad libitum and were kept on a 12:12-h light-dark cycle (light onset at 0630) at 22 ± 1°C ambient temperature for at least 1 wk of habituation before surgery and during the postsurgical recovery period. For habituation purposes, animals spent at least 1–2 h each day in a whole body plethysmograph chamber.

Ventilatory and cardiovascular recordings. Cardiorespiratory measures were continuously acquired in the freely behaving animal placed in a previously calibrated 3-liter barometric chamber (Buxco Electronics, Troy, NY), with the use of the methods described by Bartlett and Tenney (1) and Pappenheimer (19). To minimize the long-term effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size in which temperature was measured by using a T-type thermocouple was used. In addition, as previously recommended by Epstein and colleagues (6), a correction factor was incorporated into the software routine to account for inspiratory and expiratory barometric asymmetries. Environmental temperature was maintained slightly below the thermoneutral range (24–
The calibration volume of 0.5 ml of air was repeatedly introduced into the chamber before and on completion of recordings. At least 60 min before the start of each protocol, animals were allowed to acclimate to the chamber, in which humidified air (90% relative humidity) was passed through at a rate of 8 l/min by using a precision-flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes were measured by using a high-gain differential pressure transducer (Validyne, model MP45–1; Ref. 5). Analog signals were continuously digitized and analyzed on-line by a microcomputer software program (Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. Tidal volume (VT), respiratory frequency (f), and minute ventilation (V˙E) were computed and stored for subsequent off-line analysis.

Protocol. Animals received 2 ml ip of 1:5 DMSO-normal saline (vehicle). Thirty minutes later, animals were randomly assigned to an initial 10-min exposure to either room air (−O₂ protocol) or 100% O₂ (+O₂ protocol). Fifteen minutes after completion of this exposure, rats were subjected to a 15-min hypoxic challenge (10% O₂-5% CO₂-balance N₂). Animals were returned to room air for 60–90 min and then underwent a second exposure to the aforementioned protocol. In this second run, animals received the prehypoxic run gas mixture they had not been administered during the earlier run (−O₂ or +O₂). Such recovery period was previously ascertained during pilot studies to yield reproducible HVR. Two days later, rats were subjected again to an identical −O₂ and +O₂ or vice versa protocol but were pretreated before each arm of this protocol with the selective nNOS inhibitor 7-nitroindazole (7-NI; 25 mg/kg ip; RBI, Natick, MA). This dosage for central nervous system nNOS inhibition has been previously validated in the rat (4, 14). Experiments were always conducted at similar times of the day, between 0900 and 1500.

Measurement of blood-gas values. Arterial blood samples were obtained from the implanted arterial catheter. After withdrawal of 75–100 µl of blood in the dead space of the catheter, another 150 µl were sampled for immediate analysis of P>O₂, P>CO₂, and pH with a blood-gas analyzer (Ciba Corning, model 178). Measurements were always performed before the hypoxic gas switch and during the last minute of each hypoxic challenge.

Data analysis. Values are means ± SE. Baseline ventilation before each hypoxic run was defined as the average of ventilatory measures during the 3-min period immediately preceding the gas switch. For ventilatory challenges, mean V˙E values in 1-min bin were calculated, and the peak V˙E value of the hypoxic run was considered as representative of the HVR. In general, peak V˙E occurred between 9 and 12 min of hypoxic-gas switch. Statistical significance of the difference in data between −O₂ and +O₂ for each treatment group was assessed by paired t-tests. Differences in data between the two treatment groups (control and 7-NI) were compared by two-way ANOVA and the Newman-Keuls test (23). A P value <0.05 was considered statistically significant.

RESULTS

On the control treatment day, prior exposure to 100% oxygen (i.e., +O₂) did not modify baseline V˙E but was associated with significant enhancements of the peak V˙E response (Table 1; Figs. 1 and 2). Whereas V˙E increased by 60.4 ± 5.5% during hypoxia in the −O₂ protocol, prior exposure to 100% O₂ (+O₂) did not modify baseline V˙E but was associated with significant enhancements of the peak V˙E response (Table 1; Figs. 1 and 2).

Table 1. Ventilatory changes in room air- and hypoxia-exposed 17 waking, unrestrained rats treated with vehicle or 7-NI before (−O₂) and after (+O₂) a short hypoxic exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Room Air Exposure</th>
<th>10% O₂-5% CO₂ Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vr, ml/min</td>
<td>f, breaths/min</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−O₂</td>
<td>121.3 ± 4.9</td>
<td>96.1 ± 3.4</td>
</tr>
<tr>
<td>+O₂</td>
<td>114.1 ± 4.8</td>
<td>89.2 ± 2.7</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>7-NI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−O₂</td>
<td>113.7 ± 3.4</td>
<td>91.5 ± 1.9</td>
</tr>
<tr>
<td>+O₂</td>
<td>113.8 ± 4.4</td>
<td>89.9 ± 1.9</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Vr, minute expiratory ventilation; f, breathing frequency; Vr, tidal volume; 7-NI, 7-nitroindazole; NS, not significant.
group, in +O₂, peak \( \dot{V}E \) responses increased by 97.3 ± 11.4% (P < 0.005, ANOVA).

Arterial blood gases drawn during the last minute of the hypoxic challenge showed higher pH and lower arterial PCO₂ in +O₂ compared with −O₂ animals (P < 0.04; Table 2).

Administration of 7-NI did not modify \( \dot{V}E \) responses during either the +O₂ or the −O₂ runs. However, as previously shown with another nNOS inhibitor (10), 7-NI administration was associated with significant, although transient, changes in VT and f. During the initial 15 min after 7-NI injection, f increased from 90.2 ± 2.3 to 98.7 ± 2.7 breaths/min (P < 0.01) and VT decreased from 1.32 ± 0.03 to 1.23 ± 0.03 ml (P < 0.03). A gradual return to baseline values occurred over the ensuing 15 min, and no further differences relative to control conditions were observed during the +O₂ and −O₂ runs.

Inhibition of nNOS with 7-NI completely abolished the augmentation of peak \( \dot{V}E \) response previously demonstrated after the 10-min hyperoxic exposure (Figs. 1 and 2; Table 1; 7-NI vs. control: P < 0.001). Indeed, peak \( \dot{V}E \) responses were similar after +O₂ (69.5 ± 11.1%) and −O₂ (64.4 ± 8.3%). Furthermore, arterial blood gases were virtually identical during the last minute of the 15-min hypoxic challenge in the +O₂ and −O₂ runs (Table 2).

The major findings of the present study are that a short hyperoxic exposure before a hypoxic challenge leads to a significant increase of HVR in the conscious, freely behaving rat. In addition, our data suggest that nNOS may play an important role in such hyperoxia-induced HVR augmentation.

The hyperventilation induced by hyperoxia was originally reported by Miller and Tenney (16) in waking, carotid-deafferented cats. Several years later, Gautier and colleagues (8) showed that this ventilatory facilitation was most probably of central origin, since it was present in conscious cats but was abolished by anesthesia. Hyperoxic hyperventilation was also demonstrated in normal human subjects (2) as well as in patients with the Prader-Willi syndrome, who have absent peripheral chemoreceptor function (9), further suggesting a centrally mediated mechanism. Although it is unclear whether such centrally mediated hyperoxic ventilatory facilitation bears any relation to the mechanisms underlying the enhancement of HVR by previous hyperoxic exposure, such earlier reports of hyperoxic hyperventilation catalyzed the basic concept that hyperoxia may alter tonic outputs of central respiratory drives.

The favored mechanism proposed by Honda and colleagues to explain hyperoxic hyperventilation entailed the release of an excitatory agent by hyperoxia (13). These investigators further postulated that the excitatory agent was glutamate, since they found increased glutamate plasma levels in +O₂ runs as well as the presence of a linear correlation between the magnitude of HVR augmentation and glutamate concentrations. We found that when nNOS-dependent NO was blocked by 7-NI, HVR potentiation was abolished. Our experiments and those of Honda and colleagues can be reconciled within the framework of a putative glutamate-NO pathway of synaptic excitability (3, 7). Indeed, activation of N-methyl-D-aspartate glutamate receptors, which induce calcium elevations either by opening of a voltage-dependent calcium channel or by the release of intracellular calcium stores, will lead to calmodulin binding and NOS activation. NO tissue elevations will, in turn, influence neurotransmitter release either through activation of cGMP-dependent

### Table 2. Arterial blood gases in room air- and 15-min hypoxia-exposed 17 waking, unrestrained rats treated with vehicle or 7-NI before and after a short hyperoxic exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Room Air Exposure</th>
<th>10% O₂-5% CO₂ Exposure</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( \text{pH} )</td>
<td>( \text{PaCO}_2 )</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−O₂</td>
<td>7.432 ± 0.006</td>
<td>34.8 ± 1.5</td>
</tr>
<tr>
<td>+O₂</td>
<td>7.436 ± 0.007</td>
<td>33.8 ± 1.6</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>7-NI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−O₂</td>
<td>7.421 ± 0.009</td>
<td>34.7 ± 1.8</td>
</tr>
<tr>
<td>+O₂</td>
<td>7.422 ± 0.009</td>
<td>34.9 ± 2.1</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( \text{PaCO}_2 \) and \( \text{PaO}_2 \), arterial PCO₂ and PO₂, respectively.
protein phosphorylation cascades or by rapid diffusion to the presynaptic neuron to further enhance glutamate release, thus essentially behaving as a retrograde messenger (18, 21). Hyperoxia could lead to enhanced nNOS activation and increased NO release, since this enzyme exhibits O2 dependency (21). Increased tissue NO availability would, in turn, exert a previously demonstrated excitatory effect in respiratory neural regions (12, 20), thereby underlying the hypoxic potentiation that followed hyperoxia.

Several technical issues of these experiments deserve some comment. First, the overall magnitude of the ventilatory response to hypoxia in these experiments was less than that previously found when a similar hypoxic stimulus was used (12). Although we remain uncertain as to the cause(s) leading to such difference, we believe they may be accounted for, at least in part, by different commercial breeder sources. Second, we did not specifically examine the late phase of hypoxia in present experiments. This decision was based on the fact that HVR augmentation with prior exposure to hyperoxia was not associated with any changes in the magnitude of the hypoxic roll-off in humans (13). Furthermore, we wished to avoid potentially confounding effects of centrally mediated hypoxic inhibition associated with longer hypoxic exposures, which in addition are potentiated by nNOS blockade (12). Third, we employed a CO2-containing gas mixture to attempt and maintain isocapnia as closely as possible without interfering with animal behavior. As shown in Table 2, although this strategy did not achieve as close an arterial Pco2 control as might be obtained by using other techniques, such as end-tidal forcing, the values of arterial Pco2 remained within an acceptable “isocapnic” range.

In summary, I have shown that the HVR potentiation elicited by a previous short hyperoxic exposure exhibits NO-dependent behavior. The findings further underscore the physiological importance and long-lasting effects of a priori minimal perturbations when aiming to determine the magnitude and response characteristics of the ventilatory response to a defined stimulus.

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