Phenotypic variation in cardiovascular responses to acute hypoxic and hypercapnic exposure in mice

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HYPOXIA IS A POWERFUL STIMULUS to the physiological systems that regulate the cardiovascular system. During hypoxia, the body attempts to maintain an adequate blood flow and supply of oxygen to the heart and brain, while decreasing blood supply to other organs and mobilizing blood from the splanchnic circulation (2). Consequently, mean systemic arterial blood pressure (PSA) is normally maintained or slightly elevated during hypoxia, whereas heart rate (HR) tends to decrease (7). Recent studies in rats support the concept that variation in genetic background can impact on the pattern of cardiovascular responses to hypoxia. For example, Wistar-Kyoto and Sprague-Dawley rats exposed to hypoxia both exhibit brady-

ventilation; hypotension; apnea; hemodynamics; arrhythmia

METHODS

Animals. Adult, male mice (n = 6–12 per strain; 12–16 wk of age) were purchased from Jackson Laboratories and housed in a microisolator facility (AAALAC approved). The following strains were examined: A/J, BALB/cJ, C3H/HeJ, C57BL/6J CBA/J, DBA/2J, and FVB/J. Temperature and humidity were continuously regulated to 20–22°C and 40–60% RH, respectively. Food and water were available ad libitum throughout the study. Protocols were conducted on the seven strains concurrently to minimize bias. All studies were conducted with the approval of the Johns Hopkins Animal Care and Use Committee.

Surgical preparation. All surgical procedures were performed under 1–2% isoflurane anesthesia using aseptic techniques. Electrodes for polysomnography (PSG) were implanted as previously described (3). Briefly, three Teflon-coated wires were inserted into predrilled holes in the left frontal and left and right parietal regions. Two electromyographic (EMG) electrodes were stitched flat onto the surface of the muscle immediately posterior to the dorsal area of the mouse skull.

The femoral artery was exposed by a 1.5-cm cutaneous incision and carefully separated from the femoral vein and nerve, as previously described (3) for determination of systemic arterial pressure (PSA). A 60-cm-long Renathane catheter (MRE025, Braintree Scientific), heat-stretched and formed into a J-shape, was inserted with the aid of a 26-gauge needle and advanced ~0.5–1.0 cm toward the bifurcation of the aorta. The catheter was secured by suture and cyanoacrylate glue (QuickTite super glue; Manco, Avon, OH), then exteriorized at the base of the skull and secured to the EEG/EMG electrodes. The catheter was attached to a single-channel fluid swivel (375/25, Instech Laboratories) and perfused slowly by an infusion pump (0.5 ml/day) with a sterile saline solution containing heparin (80 U/ml). PSA measurements were facilitated by a flow-through pressure transducer.
Monitoring and data analysis. Both the PSG leads and the flow-through transducer were connected to a pen recorder during gas exposures (Grass Instruments, Quincy, MA). Data from the pen recorder were sampled at 300 Hz, converted to digital format (DI-200 data acquisition board; Dataq Instruments, Akron, OH), and acquired to optical disks for storage with Windaq/200 acquisition software (Dataq Instruments). PSA and HR were determined from signals averaged over 24 h.

Gas exposure protocol. All exposures were conducted at ~30 M above sea level; thus ambient pressure approximated 760 mmHg. Mice were placed in a cylindrical exposure chamber (0.7 l) with standard bedding. This chamber allowed free movement of the mouse but was small enough to enable rapid exchange of gases. Room air (FIO2 = 0.209) was forced through the chamber at 2 l/min. Mice were exposed to three different gases: 1) hypoxia (10% O2, 90% N2), 2) hypercapnia (5% CO2, 40% O2, 55% N2), and 3) combined hypoxia/hypercapnia (10% O2, 5% CO2, 85% N2). Oxygen levels in the chamber were continuously recorded (SensorMedics oxygen analyzer, model OM-11).

The exposures began after a 1-h period of acclimation to the exposure chamber. In all mice the PSG was monitored during gas exposures to ascertain wakefulness. No exposures were conducted while mice were sleeping. Each exposure lasted 4 min, with 8 min of recovery time following different exposures (i.e., between hypercapnia and hypoxia) and 4 min of recovery between repetitions of the same exposures. Each mouse was exposed to a minimum of two repetitions of each gas challenge. We have previously published baseline PSA data for many of the strains across sleep/wake states (3).

An arterial blood gas sample (80–100 μl) was obtained under room air conditions 30 min after completion of the final gas challenge and immediately analyzed on a blood gas analyzer (model IL BG3; Instrumentation Laboratory, Lexington, MA). The blood loss was replaced with an infusion of 100–200 ml of saline. On subsequent days, arterial blood samples were obtained after 4-min exposure to the...
hypoxic, hypercapnic, and combined hypoxic/hypercapnic exposures detailed above. The order of sampling was determined by block design and continued at 24-h intervals for as long as the catheter remained patent.

Statistics. Measurements of $P_{SA}$ and HR were averaged over 1-min periods throughout the 4-min exposure period and compared with a control period consisting of the 2-min interval immediately preceding the start of the gas exposure. Values (means ± SE) for $P_{SA}$ and HR were determined for each strain during the control period and at 1-min intervals during the gas exposure period. Statistical differences in cardiovascular parameters during exposure to hypoxia and hypercapnia within each strain were assessed by within-subject, one-way ANOVA, and if the ANOVA was significant, then a Dunnett post-hoc test was used to determine whether there was a change in $P_{SA}$ or HR at any time point during gas exposure relative to the control period. Statistical differences in the change in $P_{SA}$ between strains was determined by between-subject, one-way, ANOVA, and if the ANOVA was significant, then the Scheffe method post-hoc test was used to identify which strains were significantly different.

RESULTS

Hypoxia. Exposure to 10% $O_2$ induced varying levels of hypotension in most strains, with DBA/2J mice displaying the most extreme decrease in $P_{SA}$ (~30 mmHg). Figure 1 demonstrates a typical tracing from a DBA/2J mouse; note severe

Fig. 3. Individual responses for change in mean $P_{SA}$ during exposure to hypoxia (10% $O_2$; top), hypercapnia (5% $CO_2$; middle), and combined hypoxia and hypercapnia (10% $O_2$ and 5% $CO_2$; bottom) in seven inbred strains. Values represent differences between control and the 4th minute of challenge. • Statistical differences from DBA/2J strain. # Statistical differences from C57BL/6J strain. † Statistical differences from FVB/2J strain.
periods of arrhythmia-induced pulse deficit (downward deflections) in the DBA/2J mouse consequent with hypotension. Other strains (A/J, C3H/HeJ, C57BL/6J, and CBA/J) exhibited a moderate hypotension (8–16 mmHg below control), while BALB/cJ and FVB/J strains showed no significant change from baseline (data summarized in Figs. 2 and 3). The HR changes appeared unrelated to alterations in P_{SA}, with HR increasing in BALB/cJ and C3H/HeJ mice, decreasing in DBA/2J and FVB/J mice, and not significantly changing in A/J, C57BL/6J, and CBA/J mice (Fig. 4).

DBA/2J mice were particularly sensitive to hypoxia, demonstrating the most severe hypotension and bradycardia to the point of complete atrioventricular (AV)-node conduction block. Arrhythmias were frequently observed in these mice, with the most severe occurring in the final 2 min of the hypoxic exposure (Fig. 5). Arrhythmias (defined as a >50% difference in pulse interval from the preceding interval) were characterized electrocardiographically in a separate group of DBA/2J mice (n = 4) and consisted primarily of bradycardic slowing and type II AV-node blockade, indicated by presence of P-waves without ventricular deflections.

Hypercapnia. During exposure to 5% CO_{2}, all strains demonstrated slight increases in P_{SA} (Figs. 2 and 3). The only significant difference between strains occurred between the A/J and DBA/2J strain (Fig. 3). HR values decreased concomitantly in all strains, presumably due to baroreflex modulation (Fig. 4). No hypercapnia-related increase in arrhythmogenesis was observed in any strain (Fig. 5).

Combined hypoxia and hypercapnia. When administered concomitantly, the individual effects of hypoxia and hypercapnia appeared to negate each other, resulting in little or no net P_{SA} change from baseline in most strains (Fig. 2 and 3). The FVB/J mouse demonstrated the most pronounced increase in P_{SA} and was the only strain in which the hypertensive effect of combined hypoxia and hypercapnia was greater than that of hypercapnia alone (P = 0.02 by paired t-test). HR responses were variable, although bradycardia was the predominant response in most strains (Fig. 4). No significant incidence of arrhythmia was associated with combined hypoxia and hypercapnia in any strain (Fig. 5).

Blood gases. Although each strain was exposed to identical inspired levels of hypoxia and hypercapnia, it is possible that differences in blood levels of P_{A\text{O}_{2}} and P_{A\text{CO}_{2}} were in part responsible for the cardiovascular differences we observed between strains. Our intention was to measure blood gas levels during room air breathing and during hypoxia, hypercapnia, and combined hypoxia and hypercapnia exposure in each animal. However, due to the variable period of time that catheters remained patent and the requirement of a minimum period of 24 h between repeat samples to minimize blood loss, it was not possible to obtain comprehensive measurements in all strains, and subsequently the data have been pooled across strains in Table 1. There was, however, no indication from the data we did collect of any major differences between strains with respect to blood gases during hypoxic and hypercapnic exposure.

DISCUSSION

In the current study we utilized a variety of mouse strains with chronically implanted arterial catheters to determine whether genetic background influences the cardiovascular responses to acute periods of hypoxia and hypercapnia. We observed remarkable homogeneity in the P_{SA} and HR responses to hypercapnia across all seven strains, suggesting that cardiovascular responses to hypercapnia, which are predominantly mediated by central chemoreceptor activation of peripheral sympathetic nerve activity (8), are relatively resistant to
genetic influences. In contrast, there was marked variation between strains in the cardiovascular response to hypoxia, and to combined hypoxia/hypercapnia. In the discourse that follows, we examine possible reasons for the heterogeneity of cardiovascular responses to hypoxia and discuss the unique responses in the FVB/J strain and the DBA/2J strain.

Heterogeneity of responses to hypoxia and combined hypoxia/hypercapnia. The cardiovascular responses to hypoxia and combined hypoxia and hypercapnia varied considerably in terms of both the magnitude and direction of response. A number of genetic factors ranging from ventilatory response, which determines the level of PaO₂ and PaCO₂ in the arterial blood, through to autonomic output to the heart and peripheral blood vessels, could contribute to this observed variability in cardiovascular responses to hypoxia and hypercapnia.

Exposure to hypoxia or combined hypoxia/hypercapnia caused an opposite pattern of cardiovascular responses between some strains. For example, during combined hypoxia/hypercapnia, HR increased in the C3H/HeJ strain but decreased in the CBA/J strain. Despite this opposite response in HR, arterial pH, PaCO₂, and PaO₂ levels during exposure to 10% O₂ and 5% CO₂ were similar between C3H/HeJ (7.34 ± 0.03 U, 40.5 ± 1.8 mmHg, 50.0 ± 3.3 mmHg, respectively; n = 4) and CBA (7.36 ± 0.02 U, 39.8 ± 1.1 mmHg, 53.0 ± 2.7 mmHg; n = 4) animals. Thus it is unlikely that the differences in cardiovascular responses to either hypoxia or combined hypoxia/hypercapnia are solely dependent on blood gas variability between strains.

Assuming that arterial blood gas levels and arterial oxygen disassociation curves were comparable during the various gas challenges, genetic variation between strains may therefore be dependent on the chemoreceptor-autonomic reflex arc. The uniformity of cardiovascular responses to hypoxia, however, suggests that neural pathways from the central chemoreceptors, through central integration to peripheral autonomic output, are resistant to genetic variation. The same may not be true for hypoxic chemoreceptors and their subsequent activation of neural reflexes controlling sympathetic output to the heart and peripheral vessels. Indeed, we have shown that structure and function of the carotid body, the dominant hypoxic chemoreceptor, can be markedly different between strains (12). Thus variation in the carotid body or the downstream components of the neural reflex arc controlling HR and vascular tone may significantly contribute to the genetic differences in cardiovascular responses between strains exposed to hypoxia or hypoxia/hypercapnia.

Another possible mechanism contributing to cardiovascular differences between strains is the ability of vascular resistance vessels to vasodilate in response to hypoxia. The ability of hypoxia to locally decrease vascular resistance through adenine, prostaglandin, or nitric oxide pathways is considerable in systemic vessels (9). For example, in a canine model of obstructive sleep apnea, the acute hypertensive response that occurs during a period of experimentally induced airway obstruction is reversed to a pronounced hypotensive response.

Table 1. Blood gas values for all strains following the hypoxic, hypercapnic and combined challenges.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10% O₂</th>
<th>5% CO₂ + 40% CO₂</th>
<th>10% O₂ + 5% CO₂</th>
</tr>
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<tr>
<td>pH</td>
<td>7.42±0.03</td>
<td>7.52±0.04*</td>
<td>7.33±0.02*</td>
<td>7.36±0.04*†</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>32.1±4.1</td>
<td>22.2±4.2*</td>
<td>44.3±2.0*</td>
<td>40.3±2.9*†</td>
</tr>
<tr>
<td>PaO₂</td>
<td>85.9±8.9</td>
<td>39.7±4.9*</td>
<td>131.7±12.3*</td>
<td>51.9±5.2*†</td>
</tr>
</tbody>
</table>

Values are pooled means ± SE. *Significant difference from control. †Significant difference from hypoxia (10% O₂) alone, P < 0.05.
after chemical blockade of the autonomic nervous system (10). Furthermore, the fall in blood pressure after autonomic blockade is eliminated when airway obstruction is induced in the presence of hyperoxia. Thus it is possible that the variation between strains in the P_Sa responses to hypoxia and combined hypoxia/hypercapnia in the present study is in part dependent on genetic differences in local hypoxic vasodilation.

Finally, there was no evidence that differences in baseline P_Sa contributed to the variability of responses to hypoxia. In a previous study, we observed significant strain-dependent differences in baseline 24-h P_Sa, as well as differences in P_Sa modulation during REM and non-REM sleep stages (3). It is possible that differences in baseline P_Sa between strains can affect the magnitude and direction of the cardiovascular responses during acute exposure to hypoxia and hypercapnia. Correlational analysis revealed that baseline P_Sa from the awake mice in the current study had no relationship with either the direction or magnitude of P_Sa responses to any gas challenge. Furthermore, values determined from 24-h baseline P_Sa in the previous study (3), which were lower but similar in pattern to those obtained during wakefulness in the present study, also bore no correlation to the cardiovascular responses to gas challenge. Therefore, the variability in cardiovascular responses to hypoxia and combined hypoxia/hypercapnia is more likely dependent on factors such as genetic differences in acute vasodilatory responses to hypoxia than to chronic regulatory control of baseline P_Sa.

Hypoxic responses in FVB/J and DBA/2J mice. The two extremes of cardiovascular responsiveness to hypoxic exposure occurred in FVB/J and DBA/2J mice. Of all the strains examined, the FVB/J strain demonstrated the least change in peripheral vascular resistance during hypoxia, as suggested by the maintenance of P_Sa without increases in HR. Furthermore, the FVB/J strain showed the largest hypertensive response to combined hypoxia and hypercapnia. The pattern of cardiovascular responses to hypoxia seen in the FVB/J strain most closely resembles that reported in humans (4) and may provide an important model for future research. The absence of a significant vasodilatory response in the FVB/J mice may be related to a reduced sensitivity in adenosine, prostaglandin, or nitric oxide pathways that mediate local hypoxic vasodilation (9). Thus the FVB/J is an interesting and clinically relevant strain to further dissect the contributions of reflex-mediated sympathetic nerve activity and local hypoxic vasodilation in the blood pressure response to hypoxia.

An unusual response observed in the current study was the marked bradycardia during hypoxia exhibited in DBA/2J mice. One possible explanation is that the arrhythmias resulted from transient heart block due to stimulation of chemoreceptors or extracardiac bronchopulmonary reflexes (5). The previous demonstration that the DBA/2J strain has a large and sensitive carotid body (12) and the largest ventilatory response to hypoxia of several inbred strains (11) provides support for pulmonary afferents as a mediator of the profound bradycardia. However, there was no significant presence of arrhythmias in DBA/2J mice during combined hypoxic and hypercapnic exposure, when ventilation and activation of bronchopulmonary reflexes will be considerably higher than during hypoxia alone (11). Although it may be that hypercapnic activation of sympathetic pathways antagonizes the vagally mediated bradycardia, it is unclear from the present study whether the marked arrhythmia in DBA/2J mice during hypoxia is due to reflex modulation of cardiac vagal activity or perhaps represents a direct effect of hypoxia on the rhythmicity of the heart. Either way, the cardiovascular response to hypoxia in the DBA/2J strain provides an intriguing model for further study.

Summary. The cardiovascular response to hypercapnia was remarkably homogeneous between inbred strains. In contrast, hypoxia caused a heterogeneous response between inbred strains in which blood pressure was maintained or fell precipitously and HR increased, decreased, or remained unchanged. Although we did not investigate the relative contribution of arterial blood gas levels, sensory reflex arcs, or effector output mechanisms as mediators of the heterogeneous cardiovascular responses to hypoxia, our study provides relevant data for any future study assessing cardiorespiratory responses using inbred mouse strains. Moreover, we have also identified that the FVB/J strain closely models human cardiovascular responses to acute hypoxia and hypercapnia and that the DBA/2J strain demonstrates a unique cardiac susceptibility to acute hypoxic exposure.

GRANTS

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REFERENCES