Genetic background affects cardiovascular responses to obstructive and simulated apnea

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Iiyori, Nao, Machiko Shirahata, and Christopher P. O’Donnell. Genetic background affects cardiovascular responses to obstructive and simulated apnea. Physiol Genomics 24: 65–72, 2005. First published October 25, 2005; doi:10.1152/physiolgenomics.00203.2005.—We have recently demonstrated that genetic background significantly impacts the blood pressure and heart rate response to hypoxia (Campen MJ, Tagaito Y, Li J, Balbir A, Tankersley CG, Smith P, Schwartz A, and O’Donnell CP. Physiol Genomics 20: 15–20, 2005). Because hypoxia is considered a mediator of the acute and chronic cardiovascular complications of obstructive sleep apnea, we investigated whether genetic factors also influence the cardiovascular response to experimentally induced obstructive apnea (OA) and simulated apnea (SA). In three strains of inbred mice (C57BL/6J, DBA/2J, and FVB/J) anesthetized with urethane (1.2 g/kg), apnea was induced at end-expiration for 5- and 10-s periods in spontaneous breathing (OA) and mechanically ventilated (SA; pancuronium, 0.2 mg/kg bolus + 0.003 mg·kg⁻¹·min⁻¹) animals before and after administration of an autonomic ganglionic blocker (hexamethonium, 20 mg/kg). In contrast to our previous findings with hypoxia, OA produced a marked hypertensive response in all three strains. However, strain impacted on the degree of bradycardia during OA, which was large in C57BL/6J and FVB/J mice and effectively absent in DBA/2J mice. In C57BL/6J but not FVB/J mice, the bradycardia was abolished with SA under mechanical ventilation. Cardiovascular responses to SA in all strains were eliminated by autonomic blockade. These data show that 1) DBA/2J mice, in contrast to the previous demonstration of marked bradycardia during hypoxia, unexpectedly do not produce bradycardia during apnea; 2) C57BL/6J mice exhibit a bradycardia that is dependent on input from thoracic afferents; and 3) FVB/J mice exhibit a bradycardia despite the loss of thoracic afferent input, consistent with a potent pressure response eliciting a baroreceptor-mediated bradycardia. Thus genetic background can affect both the pattern and magnitude of the cardiovascular response to apnea.

Blood pressure; bradycardia; inbred mouse; thoracic afferents

The repetitive cycle of airway obstruction that occurs during sleep in patients with obstructive sleep apnea causes acute cardiovascular disturbances and increases the risk of systemic hypertension, ventricular hypertrophy, myocardial infarction, and stroke (6, 12, 14, 16). Each period of nighttime obstructive apnea (OA) causes acute periods of hypertension and bradycardia that rapidly reverse when breathing is restored between obstructive episodes (3, 9, 20). The severity of the acute hypertension and bradycardia that occur in response to OA may have an impact on the magnitude of cardiovascular morbidity and mortality that develop in patients with chronic obstructive sleep apnea. Because human patients with obstructive sleep apnea exhibit considerable variability in the acute cardiovascular response to apnea, in particular the degree of bradycardia (10, 21), it is possible that genetic background plays a significant role in the severity of the response.

We have previously shown that the cardiovascular responses to acute hypoxic and hypercapnic exposure during spontaneous breathing vary considerably between different inbred strains of mice (2). Because hypoxia and hypercapnia occur in response to apnea, we propose that genetic factors may also influence the cardiovascular response to apnea. However, the cardiovascular response to apnea is dependent not only on the stimulation of chemoreceptors by hypoxia and hypercapnia, but also on baroreceptors and thoracic afferent inputs (8, 11, 13, 17, 19). Indeed, it has been previously shown in humans that the increase in sympathetic nerve activity during exposure to combined hypoxia and hypercapnia is exacerbated in the presence of simultaneous voluntary apnea (18). It is currently unknown whether the interaction between chemoreceptors, baroreceptors, and thoracic afferent inputs during periods of apnea is dependent on genetic variability.

The purpose of the current study was to assess the cardiovascular response to OA during spontaneous ventilation and to simulated apnea (SA) during mechanical ventilation in three inbred strains of mice: C57BL/6J, DBA/2J, and FVB/J. These strains were chosen on the basis of their previously described divergent cardiovascular responses to acute hypoxic and hypercapnic exposure (2). We hypothesized that J) genetic background affects the cardiovascular responses to apnea, and 2) thoracic afferent inputs alter the neurally mediated cardiovascular response to apnea based on genetic background.

METHODS

Experiments were conducted in C57BL/6J, DBA/2J, and FVB/J inbred strains of male mice (aged 8–12 wk, n = 8 per strain; Jackson Laboratories, Bar Harbor, ME). Mice were housed in a 12:12-h light-dark (lights on at 0900), temperature-controlled environment (21–24°C) with free access to standard laboratory rodent chow and water. All experiments in the study were approved by Johns Hopkins University Animal Use and Care Committee and complied with the American Physiological Society Guidelines.

Surgical Procedures

Anesthesia was induced with inhalation of isoflurane and maintained with intraperitoneal injection of 1.2 g/kg urethane and local injection of 1% lidocaine. Supplemental doses of urethane were given intravenously to maintain a stable plane of anesthesia throughout the experiment. After induction of anesthesia, atropine (10 μg) was administered intramuscularly to inhibit secretions in the respiratory tract. Body temperature was monitored via a rectal thermistor probe and maintained at 37°C with a warming lamp.
Table 1. General experimental characteristics of all mice studied

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age, days</th>
<th>Body wt g</th>
<th>Preparation Time, min</th>
<th>Total Dose of Urethane, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J (n = 8)</td>
<td>72 ± 3</td>
<td>28.1 ± 0.7</td>
<td>151 ± 9</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>DBA/2J (n = 8)</td>
<td>83 ± 5</td>
<td>25.8 ± 0.9*</td>
<td>149 ± 8</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>FVB/J (n = 8)</td>
<td>80 ± 2</td>
<td>28.8 ± 0.6</td>
<td>134 ± 12</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preparation time was defined as the period from the induction of anesthesia through the end of catheterization. Significant differences between strains were determined by 1-way ANOVA and Tukey’s post hoc analyses. *P < 0.05 for DBA/2J mice vs. C57BL/6J mice and FVB/J mice.

After cannulation of the trachea, catheters were inserted into the femoral artery and vein. A polyethylene catheter (PE-50; Becton Dickinson) was inserted into the left femoral artery for monitoring arterial pressure and then continuously infused with heparinized saline to maintain patency (20 U/ml, 7.8 µl/h). A polyurethane catheter (model MRE025, Braintree Scientific) was placed in the left femoral vein for injection of drugs and locked with heparinized saline.

**Apparatus and Methods of Measurement**

Mean arterial pressure (MAP), heart rate (HR), ECG, and airway pressure were recorded throughout the experiment. The MAP was measured via a pressure transducer (Cobe, Lakewood, CO) zeroed at midthoracic level that was calibrated at the start and end of each experiment. The ECG was continuously monitored, and HR was displayed from the arterial pressure pulse waveform by a tachometer. Airway pressure was monitored via a pressure transducer (Cobe) from a T-branch on the tracheal tube. A pen recorder (Grass Instruments, Quincy, MA) was used to record MAP, HR, ECG, and airway pressure. The signals from the pen recorder were digitized at 500 Hz (DI-200 data acquisition board; Dataga Instruments, Akron, OH) and stored on optical disk with WinDaq/200 acquisition software (Dataq Instruments).

**Experimental Protocol**

After completion of the surgical preparation, animals were allowed a 30-min stabilization period. Apnea was experimentally induced at end-expiration for 5- and 10-s periods under three conditions: 1) spontaneous breathing (OA), 2) mechanical ventilation (SA), and 3) mechanical ventilation (SA) after autonomic blockade. Sufficient time was given between successive apneas for cardiovascular parameters to return to baseline values.

**Spontaneous ventilation.** OA was induced by occlusion of the open end of the endotracheal tube while the animal was spontaneously breathing under room air conditions.

**Mechanical ventilation.** Animals were paralyzed with intravenous administration of pancuronium (0.2-mg/kg bolus and maintenance dose of 0.003 mg·kg⁻¹·min⁻¹) and mechanically ventilated with room air at a respiratory frequency of 120 breaths/min and a tidal volume of 0.2 ml. The maintenance of a stable level of anesthesia was confirmed by stability of MAP, HR, and ECG waveforms. At least 10 min after administration of pancuronium, SA was induced for 5- and 10-s periods by turning the ventilator off at end-expiration.

**Mechanical ventilation and autonomic blockade.** In the final part of the experiment, SA was induced in the mechanically ventilated mice after administration of the autonomic ganglionic blocking agent hexamethonium (20-mg/kg iv bolus). Before and after ganglionic blockade, phenylephrine was injected intravenously to determine reflex changes in HR. The efficacy of autonomic blockade was verified by the absence of a reflex bradycardia in response to an acute increase in blood pressure after a bolus administration of phenylephrine (40–60 µg/kg).

**Data Analyses**

For each period of apnea, the following cardiac parameters were determined from analysis of the arterial pulse pressure waveform. **Baseline MAP and HR.** Average values for MAP from 20 cardiac cycles immediately before 5-s and 10-s periods of apnea.

**Peak MAP.** The highest MAP averaged over a 10-cardiac cycle period from the start of apnea to 3 s after apnea ended.

**Nadir HR.** The lowest HR averaged over a 10-cardiac cycle period from the start of apnea to 3 s after apnea ended.

**ΔMAP and ΔHR.** The difference between peak MAP and baseline MAP and the difference between baseline HR and nadir HR.

For periods of OA during spontaneous breathing, the number of respiratory efforts, peak negative airway pressure, baseline cardiovascular parameters, and ΔMAP and ΔHR. If the ANOVA was significant, a Tukey’s post hoc test was used for determining which strains were significantly different from each other. Within each strain, a significant difference between baseline MAP and peak MAP or between baseline HR and nadir HR was determined by Student’s t-test for paired data. Differences were considered significant if P < 0.05.

**RESULTS**

Table 1 shows that there were no differences between the three strains in their age, time of surgical preparation, or total dose of urethane, although DBA/2J mice had lower body weight than the other two strains. The DBA/2J mice also exhibited a lower baseline MAP as previously described (1) and slightly reduced baseline HR during both spontaneous and mechanical ventilation (Table 2). Under conditions of spontaneous ventilation, the number of obstructed breaths that occurred during the 5- and 10-s periods of OA was significantly fewer in the FVB/J mice than the C57BL/6J mice (Table 3 and Fig. 1).

Figure 1 shows sample traces of the blood pressure, HR, and airway pressure responses during a 10-s period of OA from one animal in each of the three strains. All three strains exhibited hypertension and increasingly negative peak airway pressure swings during OA, whereas the HR responses varied markedly between strains. The pooled data in Fig. 2 show that, during the

**Table 2. Baseline MAP and HR during spontaneous and mechanical ventilation**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Baseline MAP, mmHg</th>
<th>Baseline HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J (n = 8)</td>
<td>80.5 ± 2.7</td>
<td>612 ± 19</td>
</tr>
<tr>
<td>DBA/2J (n = 8)</td>
<td>56.5 ± 2.6*</td>
<td>548 ± 19*</td>
</tr>
<tr>
<td>FVB/J (n = 8)</td>
<td>76.9 ± 2.5</td>
<td>617 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; bpm, beats/min. Significant differences between strains were determined by 1-way ANOVA and Tukey’s post hoc analyses. *P < 0.05 for DBA/2J mice vs. C57BL/6J mice and FVB/J mice.
5-s period of OA, all three strains exhibited significant MAP responses and that the FVB/J response was greater than that of the other two strains. The FVB/J strain also had a profound bradycardia compared with the mild bradycardia seen in the C57BL/6J mice. The DBA/2J mice showed no detectable change in HR during OA and also had the smallest peak negative airway pressure swings (Table 3). During the 10-s period of OA, all three strains showed similar hypertensive responses, and the pattern of bradycardia remained similar to that seen during the 5-s period of OA, except that the DBA/2J strain now exhibited a very mild bradycardia.

**SA During Mechanical Ventilation**

The sample tracings in Fig. 3 show that mechanical ventilation effectively eliminated the bradycardic response to 10-s periods of SA in the C57BL/6J and DBA/2J strains, but only caused minor reductions in bradycardia in FVB/J mice compared with responses during spontaneous ventilation (Fig. 1). The pooled data in Fig. 4 show a similar pattern of hypertensive response to SA as that seen during OA in Fig. 2. However, during the 10-s period of apnea, the magnitude of the hypertensive response in the DBA/2J (P < 0.05) and FVB/J (P < 0.0025) mice was significantly greater during mechanical ventilation (Fig. 4) than during spontaneous ventilation (Fig. 2). In C57BL/6J mice, mechanical ventilation reduced the bradycardic response to both 5-s (P < 0.025) and 10-s (P < 0.0005) periods of apnea compared with spontaneous ventilation, such that a significant bradycardia no longer was observed during mechanical ventilation (note: both the C57BL/6J and DBA/2J strains were still capable of producing a significant bradycardic response to acute hypertension from bolus administration of phenylephrine during mechanical ventilation, as detailed in Table 4). In contrast to the C57BL/6J and DBA/2J strains, the FVB/J strain continued to exhibit a marked bradycardia in response to SA under conditions of mechanical ventilation (Figs. 3 and 4). Thus the FVB/J strain was able to produce increases in MAP in response to SA comparable to those shown in the C57BL/6J and DBA/2J strains despite the continuing presence of a profound bradycardia.

**SA During Mechanical Ventilation and Autonomic Blockade**

Administration of hexamethonium (20 mg/kg) completely blocked reflex decreases in HR during acute hypertension from bolus phenylephrine (40–60 mg/kg) administration in all three

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### Table 3. Respiratory responses to OA during spontaneous ventilation

<table>
<thead>
<tr>
<th>Duration of OA</th>
<th>No. of Respiratory Efforts, breaths</th>
<th>Peak Negative Airway Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 s</td>
<td>10 s</td>
</tr>
<tr>
<td></td>
<td>5 s</td>
<td>10 s</td>
</tr>
<tr>
<td>C57BL/6J (n = 8)</td>
<td>19.9±0.9‡ 31.4±1.4‡</td>
<td>9.2±1.4‡ 27.9±2.1§</td>
</tr>
<tr>
<td>DBA/2J (n = 8)</td>
<td>17.5±2.9 27.0±2.7 6.8±0.8† 17.4±1.9†</td>
<td></td>
</tr>
<tr>
<td>FVB/J (n = 8)</td>
<td>13.0±1.0 19.9±1.6 15.8±2.5 29.6±3.6</td>
<td></td>
</tr>
</tbody>
</table>

Value are means ± SE. OA, obstructive apnea. Significant differences between strains were determined by 1-way ANOVA and Tukey’s post hoc analyses. †P < 0.05 for DBA/2J mice vs. FVB/J mice. ‡P < 0.05 for C57BL/6J mice vs. FVB/J mice.
strains. The sample tracings in Fig. 5 show that autonomic blockade also eliminated any changes in HR associated with SA during mechanical ventilation. Small, inconsistent changes in MAP, presumably related to mechanical shifts in blood from the apnea, were observed (e.g., FVB/J mouse in Fig. 5) but were minor compared with the hypertensive responses seen before autonomic blockade (Fig. 3). Thus the hypertension and bradycardia that occurred in all three strains were dependent on neurally mediated autonomic reflexes.

DISCUSSION

The impact of experimentally induced apnea on cardiovascular responses has not been examined previously in mice. A comparative assessment of cardiovascular responses between inbred mouse strains provides insight into the potential for genetic background to influence the magnitude of hypertension and bradycardia that occur in obstructive sleep apnea. For the purposes of the current study, we chose to examine three inbred mouse strains based on their diversity of blood pressure and HR responses to hypoxia during spontaneous ventilation, as recently described (2): FVB/J mice maintain blood pressure in the presence of mild bradycardia, C57BL/6J mice exhibit hypotension and mild bradycardia, and DBA/2J mice have significant hypotension and severe bradycardia. In the current study, apnea caused acute hypertensive and bradycardic responses in all three strains of mice. However, strain signifi-

Fig. 2. Average ± SE change in mean arterial pressure (ΔMAP) and change in HR (ΔHR) during 5- and 10-s periods of OA in C57BL/6J (open bars), DBA/2J (solid bars), and FVB/J (hatched bars) mice under conditions of spontaneous ventilation. *Significant change within strain in either MAP or HR in response to OA compared with the preceding baseline period as determined by Student’s 2-tailed paired t-test. Significant differences between strains were determined by 1-way ANOVA and Tukey’s post hoc analyses: #P < 0.05, ##P < 0.005, and ###P < 0.0005.

Fig. 3. Sample tracings showing changes in arterial blood pressure, HR, and airway pressure from a C57BL/6J, DBA/2J, and FVB/J mouse during 10-s periods of simulated apnea (SA) under conditions of mechanical ventilation.
cantly affected the magnitude of the cardiovascular responses, in particular the degree of bradycardia, which was large in C57BL/6J and FVB/J mice and almost nonexistent in DBA/2J mice. In C57BL/6J mice, input from thoracic afferents contributed significantly to the severity of bradycardia, whereas the even larger bradycardia in the FVB/J strain remained present during mechanical ventilation and did not negate a substantial hypertensive response. Thus genetic background can have an impact on the acute cardiovascular responses to apnea, and in the discussion that follows we examine the mechanisms and neural pathways potentially contributing to this heterogeneity of responses between inbred strains.

Genetic Influences on the Bradycardic Response to Apnea

The presence of bradycardia during apneic episodes is a well-characterized feature of obstructive sleep apnea (10, 21) and has been demonstrated to various degrees in animal models of airway obstruction in cats (13), pigs (15), and dogs (19). The neural mechanisms that may induce bradycardia during apnea include the chemoreceptors, carotid and aortic baroreceptors, and afferent inputs from the lung, chest wall muscles, and heart. Hypoxic stimulation of the carotid body can result in tachycardia during spontaneous ventilation but can cause bradycardia in the absence of an increase in ventilation, putatively due to reflexes mediated by lung inflation (4). Inhibitory input from baroreceptors may also produce bradycardia in the presence of a significant pressor response. Thus a number of afferent inputs can potentially contribute to bradycardia during apnea, although it is unclear what determines their relative importance.

The relationship between chemoreceptors, baroreceptors, and thoracic afferents is unique during apnea. Clearly, apnea activates chemoreceptors and ventilation does not increase, but during obstructed inspiratory efforts, thoracic afferents will be stimulated due to mechanical distortion of the lung and the chest wall muscles and from volume changes of the heart due to redistribution of blood between the extrathoracic and thoracic regions. In addition, the decreases in intrathoracic pressure during each obstructed inspiration will provide an increase in transmural pressure across the thoracic aorta and heart, producing a relative loading of thoracic baroreceptors (5), independent of the overall increase in total peripheral resistance that occurs throughout the apnea (Figs. 1–4). Our data show that the obstructed respiratory efforts can impact the magnitude of the bradycardia, as seen in the C57BL/6J mouse in which the substantial bradycardic response to 10 s of OA was eliminated by the loss of input from thoracic afferents under conditions of mechanical ventilation (Figs. 2 and 4). These findings suggest that, in the C57BL/6J mouse, the stimulation of some combination of aortic baroreceptors or afferent inputs from the lung, chest wall muscles, or heart during OA can cause a significant bradycardia. However, in the DBA/2J and FVB/J strains, the magnitude of the bradycardic response to apnea had little, or no, dependence on thoracic afferents (Figs. 2 and 4). In the only other comparable experimentally induced apnea study performed in animals, Tarasiuk and Scharf (19) showed in anesthetized dogs that bradycardia was slightly greater during mechanical ventilation than during spontaneous ventilation. Taken together, these results indicate that, despite the absence of any change in ventilation, input from thoracic afferents during apnea can, in certain strains and species, modify the bradycardic response. However, the relative importance of aortic baroreceptors and thoracic afferents during mechanical ventilation.

Table 4. Baroreflex decrease in HR in response to an acute period of hypertension induced by bolus administration of phenylephrine (iv) during mechanical ventilation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Baseline MAP, mmHg</th>
<th>Baseline HR, bpm</th>
<th>Max MAP, mmHg</th>
<th>Min HR, bpm</th>
<th>MAP, mmHg</th>
<th>HR, bpm</th>
<th>ΔHR, bpm</th>
<th>ΔHR/MAP, bpm/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>81.2 ± 3.6</td>
<td>661 ± 16</td>
<td>135.9 ± 4.6</td>
<td>575 ± 34</td>
<td>54.7 ± 5.1</td>
<td>86 ± 27</td>
<td>1.50 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>DBA/2J</td>
<td>49.4 ± 3.0†</td>
<td>576 ± 233</td>
<td>113.5 ± 3.5</td>
<td>472 ± 45</td>
<td>64.1 ± 4.3</td>
<td>103 ± 31</td>
<td>1.80 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>FVB/J</td>
<td>72.3 ± 4.2</td>
<td>651 ± 11</td>
<td>137.9 ± 2.9</td>
<td>501 ± 38</td>
<td>65.7 ± 4.2</td>
<td>150 ± 33</td>
<td>2.23 ± 0.44</td>
<td></td>
</tr>
</tbody>
</table>

Values for MAP and HR were determined over 10 cardiac cycles and are reported as means ± SE. A bolus of phenylephrine (40 or 60 mg/kg iv) was administered to produce a visually detectable change in HR associated with an increase in arterial pressure. Average dose did not differ between strains (C57BL/6J, 45 ± 5 mg/kg; DBA/2J, 50 ± 4 mg/kg; FVB/J, 46 ± 4 mg/kg). Max, maximum; Min, minimum. Significant difference between strains was determined by 1-way ANOVA and Tukey’s post hoc analyses. †P < 0.001 and ‡P < 0.05 for DBA/2J mice vs. C57BL/6J and FVB/J mice.
from lung, chest wall muscles, and heart in mediating the bradycardia during OA remains to be determined.

Nevertheless, a major finding of the current study was that genetic background significantly impacts how thoracic afferent inputs modulate HR during apnea. DBA/2J mice exhibited little or no bradycardic response to apnea, in contrast to our previous results showing that, during acute hypoxic exposure with unobstructed spontaneous ventilation, this strain produced a marked bradycardic response (2). Thus the DBA/2J strain appears unique and represents an exception to the established concept that, in the presence of hypoxic stimulation, bradycardia is greater when ventilation is allowed to increase (4). Responses in the FVB/J strain were also unexpected in that the profound bradycardic response to apnea was not affected by loss of input from thoracic afferents. This suggests that the bradycardia in FVB/J mice was not dependent on an interaction between chemoreceptors and thoracic afferents but likely results from either a direct carotid body-mediated response or a baroreceptor-mediated response to the increase in MAP. Our previous study (2), referred to above, showed that during acute hypoxia in the presence of spontaneous ventilation, there was no significant change in blood pressure and a bradycardia of only 70 beats/min (bpm) in the FVB/J strain. In the current study, we show that, during mechanical ventilation, an acute increase in blood pressure of 65.7 ± 4.2 mmHg over a 5- to 10-s period from a bolus phenylephrine infusion produced a reflex bradycardia of 150 ± 33 bpm (Table 4). Given that the bradycardia observed during a 10-s SA in the FVB/J strain was in excess of 300 bpm (Fig. 4), it is likely that both carotid chemoreceptors and inhibitory input from the baroreceptors contribute to the marked bradycardia in FVB/J mice during apnea. Thus both the magnitude of the bradycardic response and the afferent neural mechanisms that mediate the responses are highly dependent on genetic background.

Genetic Influences on the Pressor Response to Apnea

All three strains exhibited an acute, marked, hypertensive response to apnea. The magnitude of the hypertensive response to apnea appears to contrast the pressor response that occurs during exposure to a combined hypoxic/hypercapnic stimulus during spontaneous breathing in previous studies using these three strains (2). During unobstructed spontaneous breathing, only FVB/J mice exhibited a pressor response (12 mmHg) to a combined hypoxic and hypercapnic stimulus, with both C57BL/6J and DBA/2J mice maintaining MAP at baseline levels during exposure. If we assume that 10 s of apnea will produce either comparable or milder blood gas disturbances than a 4-min exposure to 10% O2 + 5% CO2 during spontaneous ventilation [arterial partial pressure of O2 (PAO2) decreased 34.0 mmHg, Paco2 increased 8.2 mmHg, and pH decreased 0.06 units (2)], then altered input from thoracic afferents must play a potent role in amplifying the magnitude of the pressor response. These data are consistent with the human studies of Somers et al. (18) showing that apnea augments muscle sympathetic nerve activity during hypoxic/hypercapnic gas exposure in humans. The current study extends these previous findings by demonstrating that the mag-

Fig. 5. Sample tracings showing the absence of significant changes in arterial blood pressure and HR in a C57BL/6J, DBA/2J, and FVB/J mouse during 10-s periods of SA under conditions of autonomic blockade and mechanical ventilation.
nitude of the pressor response that occurs during apnea is dependent on genetic background. In particular, FVB/J mice exhibited a large pressor response during SA despite the presence of a profound bradycardia of >300 bpm. Assuming in mice that cardiac output is largely determined by the high baseline HR, and the effects of stroke volume on cardiac output are less important (7), then the increase in total peripheral resistance in the FVB/J mice during SA is substantial, since the rise in blood pressure is occurring in the presence of a cardiac output that has effectively halved. Thus genetic background makes the FVB/J strain susceptible to a potent pressor response during apnea associated with a marked compensatory bradycardia.

Limitations of Current Study

We were unable to measure the arterial blood gas changes that occurred in response to apnea. Given the relatively brief period of apnea (5–10 s) and the time it takes (~15–30 s) to extract 80–100 μl of blood via a Hamilton syringe through the very small lumen of the arterial catheter, it was not feasible to assess the peak blood gas changes that occur at the end of the period of apnea. Nevertheless, it is unlikely that the changes in blood gases during such a brief period of apnea would vary significantly between strains and account for the different patterns of cardiovascular response. A second concern is that baseline MAP and HR were not identical between strains, in particular for the DBA/2J strain, which exhibited a lower MAP and HR than the other two strains (Table 2). However, we have previously shown that chronically instrumented DBA/2J mice have a reduced MAP and HR during quiet wakefulness compared with either C57BL/6J or FVB/J mice (2) and over a continuous 24-h period had an average blood pressure below 90 mmHg (1). Thus the differences in baseline MAP and HR between strains in the current study are likely dependent on both genetic background and anesthesia. For this reason, we used the change from baseline in MAP and HR when we compared cardiovascular responses to apnea between the three strains. Despite any interactive effects of genetic background and anesthesia on reducing baseline blood pressure, it was evident during mechanical ventilation that the DBA/2J strain was capable of producing a marked hypertensive response to SA (Fig. 4) and a reflex bradycardia in response to a bolus phenylephrine infusion (Table 4) that was comparable in magnitude to that seen in the other two strains. These hypertensive and bradycardic responses in the DBA/2J strain demonstrate that reflex neural control of both the peripheral vasculature and heart was intact during mechanical ventilation, despite the lower baseline blood pressure and HR. Thus the complete absence of bradycardia in the DBA/2J mice during SA was not due to an inability of the cardiac nerves to slow HR but rather to a unique response to apnea that was dependent on genetic background. Finally, given the very high respiratory rates, it was not always possible during either spontaneous or mechanical ventilation to precisely obstruct the airway at end-expiration. (e.g., see Fig. 3). However, despite this limitation, cardiovascular responses within strains were remarkably consistent, and there was no evidence that the timing of apnea was impacting MAP and HR responses.

In summary, the current study shows that apnea causes acute hypertensive and bradycardic responses in all strains of inbred mice studied, but that the relative importance of specific afferent inputs in determining the pattern and severity of acute cardiovascular responses is significantly altered by genetic background. Our results in DBA/2J mice demonstrate an exception to the established concept that, during chemoreceptor stimulation, bradycardia is greater in the absence of an increase in ventilation, as occurs with apnea, than when ventilation is allowed to increase. The overall implication of our findings is that genetic factors likely contribute to the cardiovascular susceptibility of sleep apneic patients during acute episodes of airway obstruction.

REFERENCES


