Rats selectively bred for differences in aerobic capacity have similar hypertensive responses to chronic intermittent hypoxia

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Elevated muscle sympathetic nerve activity (SNA) is a consistent feature of hypertensive patients with sleep apnea, and treatment with continuous positive airway pressure decreases both SNA and mean arterial pressure (MAP) in patients with sleep apnea hypertension (41). Animal studies (11, 16) have shown a similar rise of MAP after as few as 7–10 days of chronic intermittent hypoxia (CIH), which mimics the episodes of hypoxemia that occur in individuals with sleep apnea.

Previous studies have investigated mechanisms of CIH-induced hypertension, and most have implicated an important role for the activation of SNA. Activation of sympathoexcitatory neurons in the rostral ventrolateral medulla (RVLM) appears critical, and this likely depends, at least partly, on excitatory inputs from various sources, but most notably from the nucleus tractus solitarius (NTS) neurons responsive to arterial chemoreceptors (12, 26). Another source of sympathoexcitatory drive arises from the hypothalamic paraventricular nucleus (PVN). In the PVN, CIH has been reported to increase the neuronal expression of FosB/ΔFosB (4, 22), and chronic inhibition of PVN with the GABA_A receptor agonist muscimol has been reported to largely prevent the CIH-induced rise of MAP (5). In the latter study, chronic blockade of ANG II type 1 receptors in the PVN had a similar anti hypertensive effect as muscimol. These effects appear to correspond to reduced inhibitory signaling by nitric oxide (NO) in the PVN (3) and recruitment by CIH of a sympathoexcitatory vasopressinergic PVN to RVLM pathway (20, 21).

Selected breeding of rats from a heterogeneous stock has led to the development of two strains that differ significantly in their metabolic phenotypes and aerobic endurance capacity (23): so-called low-capacity runners (LCR) and high-capacity runners (HCR). Over generations of selection, LCR rats have developed correlated traits that resemble human metabolic syndrome (i.e., increased body weight, insulin resistance, and increased plasma triglycerides) (34, 46). These selected lines provide an opportunity to examine whether intrinsic differences in aerobic capacity lead to corresponding differences in SNA and MAP responses to CIH. No study to date has compared rats with inbred divergent metabolic phenotypes for their susceptibility to CIH-induced hypertension. We, therefore, 1) compared the role of ongoing SNA in the maintenance of resting MAP in LCR and HCR rats, 2) determined if reduced aerobic capacity leads to exaggerated CIH-induced hypertension, and 3) determined the role of PVN neuronal activity in supporting SNA and elevated MAP after CIH exposure.

METHODS

Animals. Male rats (14–16 mo old) from generation 23 of LCR and HCR selectively bred lines (University of Michigan Medical School, Ann Arbor, MI) were used in this study (see Ref. 23 for a further

CARDIOVASCULAR DISEASES, including arterial hypertension, in patients with sleep apnea is prevalent among hypertensive patients, and >50% of patients with sleep apnea are clinically hypertensive (10, 32, 36). In addition, the severity of sleep apnea and severity of hypertension secondary to sleep apnea are both positively correlated with body mass index (10, 32, 33, 36). Thus, important common etiologies appear to exist between obesity, sleep apnea, and hypertension. However, it is presently unclear how or even if these conditions are mechanistically related and what steps could be taken clinically to decrease risk associated with comorbidity.
description). The initial rat founder population for selection was from heterogeneous N:NIH out-crossed stock. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of University of Texas Health Science Center (San Antonio, TX). Before the beginning of the study, rats were housed in clear Plexiglas cages with wood chip bedding. Food and water were available ad libitum throughout the study. The vivarium was kept at ~22°C and a 14:10-h light-dark cycle (lights on at 07:00 hours and off at 21:00 hours).

Surgical instrumentation. As previously described (2, 22), rats (n = 8 HCR rats and 7 LCR rats) were anesthetized with isoflurane (3% in O₂), heart rate (HR), and activity 24 h/day while rats were conscious and unrestrained. Rats were treated with buprenorphine (0.05 mg/kg) and penicillin postoperatively to prevent pain and infection, respectively. Among transmitter-instrumented rats, a subset (n = 6 rats/group) was used for ganglionic blockade experiments. These rats were fitted with an indwelling femoral venous catheter (PE-50 tubing) that was tunneled subcutaneously and exited dorsally between the scapulae. Rats were singly housed after surgery and allowed at least 10 days of recovery before the study continued.

Ganglionic blockade. On the experimental day, the transmitter of each rat was interfaced with a telemetry receiver connected to an analog output unit (model R11CPA, Data Sciences, St. Paul, MN) was implanted to monitor arterial blood pressure (ABP), heart rate, and the delivery of paralytic (gallamine triethiodide, 25 mg/kg bolus and then 20 mg·kg⁻¹·h⁻¹ infusion), respectively. After tracheal cannulation, rats were artificially ventilated with a moving average time constant of 5 s. Signals were digitized (5 kHz) and acquired using Spike2 software (version 7, Cambridge Electronic Design, Cambridge, UK). Recorded variables were allowed to stabilize for at least 45 min after surgery before the microinjection protocol was begun. After at least 10 min of stable baseline data had been recorded, the GABA_A receptor agonist muscimol was injected bilaterally into the PVN (0.1 nmol in 100 nl/side) at the following stereotaxic coordinates: 2.0 mm posterior to the bregma, 0.3 mm lateral from the midline, and 7.6 mm ventral from the dura. Fluorescent microspheres were coinjected with muscimol (0.2%) to mark the location of PVN injection sites and to estimate the distribution of injected muscimol. Responses of splanchic SNA (SSNA), HR, and ABP to PVN-injected muscimol were monitored for 60 min. At the end of each experiment, hexamethonium was given (30 mg/kg iv) to block ganglionic transmission and thereby determine noise in the integrated SSNA signal.

Data analysis and statistics. Electrical noise determined 5 min after ganglionic blockade was subtracted from all recorded SSNA voltages before statistical analysis. MAP during microinjections was calculated offline as diastolic pressure + one-third pulse pressure. HR during telemetry was determined from the ABP signal and during microinjection experiments from the ECG R-wave frequency. Brains of rats that received microinjections into the PVN were histologically examined for proper placement of injections. Body weight, baseline MAP and HR, and MAP responses to hexamethonium were each compared between HCR and LCR rats by ANOVA. Changes in MAP and HR during CIH were compared across days by two-way repeated-measures ANOVA using time and the selected line (HCR or LCR) as independent variables. Significant changes within a group were tested using one-way repeated-measures ANOVA comparing the 3-day baseline average to each of the 7 CIH days. Dunnett’s post hoc multiple-comparison test was used as appropriate to compare baseline values with daily values during CIH.

RESULTS

Comparison of baseline variables. Consistent with literature evidence (23–25, 46), the body weight of LCR rats was significantly (P = 0.0003) greater than that of age-matched HCR rats (Table 1). Table 1 also shows that whereas resting values of MAP and HR (determined as 3-day 24-h averages) were not significantly different between strains, 24-h average activity counts were significantly greater in HCR rats than in LCR rats (P = 0.006).

Table 1. Body weight and baseline values of mean arterial pressure, heart rate, and activity of HCR and LCR rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Rats/Group</th>
<th>Body Weight, g</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>24-h Activity, counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCR</td>
<td>8</td>
<td>413 ± 32</td>
<td>102 ± 1.3</td>
<td>326 ± 5</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>LCR</td>
<td>7</td>
<td>640 ± 35*</td>
<td>102 ± 1.6</td>
<td>≥ 299 ± 11</td>
<td>0.9 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. HCR, rats bred for selection of high aerobic capacity running; LCR, rats bred for selection of low aerobic capacity running. *P = 0.0003 and †P < 0.006 vs. the HCR group.
Effects of ganglionic blockade. Figure 1A shows representative responses to an intravenous injection of hexamethonium in conscious HCR (left) and LCR (right) rat. Note that both responded with an abrupt fall of MAP. The summary data shown in Fig. 1B indicate that blockade of ganglionic transmission significantly decreased MAP of both HCR and LCR rats (P < 0.0001). However, baseline MAP (P = 0.22), minimum MAP reached after hexamethonium (P = 0.17), and maximal change of MAP in response to hexamethonium (P = 0.50) were not different across groups.

Effect of CIH exposure. Figure 2A shows that compared with baseline, CIH significantly increased MAP in HCR and LCR rats (P < 0.05), both during hours of intermittent hypoxia (08:00–16:00 hours; left) and when averaged over 24 h (0:00–24:00 hours; right). There were no significant differences in the MAP response to CIH across HCR and LCR rats. As shown in Table 1, resting HR was not different in HCR and LCR rats. Figure 2B shows that during exposure to CIH (left), HR increased significantly (P < 0.05) in HCR and LCR rats on most days of CIH. When averaged across 24 h (Fig. 2B, right), HR among HCR rats increased on days 1 and 2 of CIH (P < 0.05), such that values were significantly greater (P < 0.05) than those of LCR rats. Both selected lines exhibited a circadian rhythm of activity with greater activity at night than during the day (data not shown). At baseline, 24-h activity was greater in HCR rats than in LCR rats (see Table 1), and this difference persisted throughout the CIH protocol (main effect of strain, P < 0.03; Fig. 2C, right). Although MAP increased during the nocturnal period of day when rats were exposed to intermittent hypoxia (08:00–16:00 hours), hypoxic exposures had no significant main effect on activity (Fig. 2C, left).

Effects of PVN inhibition. On the day after the completion of the 7-day CIH protocol, the GABA_A receptor agonist muscimol was bilaterally injected into the PVN of anesthetized HCR and LCR rats. Representative records of ABP, MAP, integrated SSNA, and HR from HCR (left) and LCR (right) rats are shown in Fig. 3A. Note that PVN-injected muscimol caused a prompt and gradual reduction of MAP and HR in both rats, whereas SSNA was only slightly reduced. As when rats were conscious, baseline MAP under anesthesia was not significantly different between HCR and LCR rats (HCR: 100 ± 6 mmHg, n = 4; LCR: 99 ± 9 mmHg, n = 5). The summary data shown in Fig. 3B demonstrate that PVN-injected muscimol significantly decreased MAP (P < 0.02) 30 min postinjection in both strains. The depressor effect of PVN-injected muscimol was significantly greater in HCR rats than in LCR rats (P < 0.05; Fig. 3B, left), despite similar reductions of ongoing SSNA (Fig. 3B, middle) and HR (Fig. 3B, right).
Figure 4A shows a representative photomicrograph of the distribution of fluorescent microspheres coinjected with muscimol into the PVN of an HCR rat. The summary distribution data shown in Fig. 4B indicate that injections spanned the rostral-caudal PVN in both groups and were largely confined to the dorsal and lateral portions of the nucleus bilaterally.

DISCUSSION

Rats selectively bred based on aerobic endurance have traits that are highly correlated with the initial breeding selection criteria, including body weight, O₂ utilization, plasma insulin and triglycerides, cardiovascular function, lipid versus glucose metabolism, and response to hypoxia (31, 34, 35, 40, 46). Although differences in circulating lipids, glucose, and associated hormones were not confirmed in the present study, LCR rats in the present study had significantly greater body weights than age-matched HCR rats and were less active, confirming that LCR rats have traits resembling those of human obesity and metabolic syndrome (23–25, 34, 35, 40). Thus, LCR and HCR selected lines provide a valuable model for investigating cardiovascular and metabolic disease comorbidity.

Despite phenotypic differences between HCR and LCR rats, we determined that values of resting MAP were similar across groups. This confirms previous reports (31, 35) but is contrary to a more recent study (7) where male LCR rats were reported to have slightly greater resting MAP than HCR rats. Whether the latter disparity relates to the use of different generations of rats (generation 23 vs. 27) or other differences in study design is unknown. Previously, HCR rats were reported to have increased cardiac sympathetic and parasympathetic tone compared with LCR rats (24, 31). This suggests that HCR rats have a greater dynamic range of autonomic control of cardiac function and possibly MAP. Here, we tested the role of autonomic activity in the maintenance of resting MAP and found that MAP fell from baselines in HCR and LCR rats to similar values during ganglionic blockade. This suggests that levels of parasympathetic and sympathetic tone in HCR and LCR rats result in no net difference in the autonomic contribution to blood pressure maintenance. An important caveat to this interpretation is that pressor mechanisms activated during the fall of MAP caused by ganglionic blockade could have differentially contributed to the support of MAP. Experiments in Sprague-Dawley rats argue against this possibility, as release of the pressor hormones ANG II and arginine vasopressin do not significantly alter the maximum depressor effect of hexamethonium (39).

According to literature evidence, hearts isolated (dennervated) from HCR rats generate greater cardiac output at a given preload and afterload than those of LCR rats (19). Additional studies are needed to determine if this difference in cardiac performance is maintained in intact HCR and LCR rats during ganglionic blockade. To the extent that it is, then similar values of MAP during ganglionic blockade may be more strongly...
determined by cardiac output in HCR rats and by vasoactive mechanisms in LCR rats. 

Contrary to our hypothesis, LCR rats did not develop an exaggerated hypertensive response to CIH compared with HCR rats. This indicates the lower maximal O2 utilization (46), increased susceptibility to hypoxia-induced cardiac dysfunction (35), and high levels of circulating insulin (34, 40) previously reported for LCR rats do not predispose them to develop an exaggerated hypertensive response to 7 days of CIH exposure. Although the CIH protocol used here exposed the rats to shorter duration and less severe hypoxia than have been previously used by other groups (3, 5, 8, 9, 27, 30, 44), it nevertheless resulted in a significant increase of MAP in both HCR and LCR rats. By only reducing inspired O2 to a nadir of 10%, we aimed to minimize the interruption of sleep patterns and stress of perceived asphyxia while still inducing hypertension. In this regard, it should be noted that rats were equally active (distance traveled) during the light cycle at baseline before the onset of CIH and after the CIH protocol began (Fig. 2C), suggesting that there was little disruption of normal light cycle activity induced by hypoxic episodes. While our results suggest that the initial development of hypertension in response to a 7-day exposure to CIH is not different between HCR and LCR rats, it remains to be determined whether longer periods of CIH exposure or more severe hypoxia would reveal a differential hypertensive response across HCR and LCR rats.

Still, studies have noted an impaired exercise capacity in sleep apnea patients (37, 38) and an improvement in sleep apnea symptoms and blood pressure after an aerobic exercise training regimen (1). Additionally, a strong correlation between increased body mass index is seen with severity of sleep apnea and hypertension (32, 33). The present study suggests that although hypertension associated with sleep apnea can be improved by regular exercise, an intrinsically low capacity for aerobic endurance does not predispose to the development of a greater hypertensive response to CIH, nor does an intrinsically greater aerobic capacity provide protection. Interestingly, voluntary apnea has been reported to elicit exaggerated pressor and muscle sympathoexcitatory responses in elite divers (15). Thus, an individual’s cardiovascular/autonomic response to a single bout of apnea may not be predictive of their hypertensive response to chronic episodes of apnea/hypoxia.

Confirming previous observations (16), our locomotor activity data during hypoxia suggest that rats did not significantly increase their activity during this time and reaffirms observations that intermittent hypoxia does not cause behavioral activation/arousal during the nocturnal portion of the sleep-wake cycle. An increase in HR during the hypoxic period is consistent with previous reports in rodents (18, 30) and humans (14); however, other studies have not shown a change in HR after CIH (3–6, 9, 16, 22, 28). The increase in HR in response to CIH was seen in both HCR and LCR rats, suggesting (but not proving) a similar vagal and sympathetic response between these selected rat lines.

In the present study, we observed that although CIH induced a similar hypertensive response in HCR and LCR rats, hypertension in HCR rats was more dependent on PVN neuronal activity. Experiments have established that preautonomic PVN neurons regulate SNA and arterial pressure via projections to the RVLM and the spinal intermediolateral cell column (13). Retrograde tracing from the RVLM combined with c-Fos staining has revealed that acute hypoxia activates neurons in the hypothalamic PVN (17). Repeated acute hypoxic events (as are seen in the CIH model) may lead to alterations of the PVN-RVLM circuitry and could be one mechanism mediating CIH-associated hypertension. In support of this hypothesis, our 7-day CIH protocol has been recently reported to increase FosB/ΔFosB staining in a variety of autonomic nuclei, including the NTS, PVN, and RVLM (4, 22).

Discharge of preautonomic PVN neurons is regulated by complex interactions between inhibitory (GABA, NO, etc.) and excitatory (glutamate, ANG II, etc.) inputs, with inhibition normally being dominant (29, 47). A recent report (21) has indicated that exposure to 7–10 days of CIH leads to a blunted increase of MAP in response to blockade of GABA_A receptors in PVN, which is consistent with CIH causing disinhibition of PVN. In the present study, we found that bilateral inhibition of PVN with the GABA_A receptor agonist muscimol decreased MAP in both LCR and HCR rats. This is consistent with evidence from Sprague-Dawley rats exposed to CIH (5). In this study, the depressor response to PVN-injected muscimol was greater in HCR rats than in LCR rats. This result is contrary to our hypothesis but nevertheless suggests that the hypertensive response to 7 days of CIH differs between these selected lines.

An important caveat to our PVN inhibition study is that differential depressor effects of PVN-injected muscimol between HCR and LCR rats may have existed before CIH. Because experiments were not performed in sham-treated rats breathing normoxic air, we cannot conclude with certainty that strain differences in response to PVN inhibition are due to differential responses to CIH. It is possible that the greater fall of MAP produced by muscimol in HCR rats reflects an enhanced role for PVN in maintaining elevated sympathetic tone in this strain (31). Indeed, even under normal conditions, the balance of excitatory/inhibitory tone in the PVN may be shifted further toward excitation in HCR rats compared with LCR rats. If so, and if exposure to CIH further shifts this balance toward excitation, as suggested by literature evidence (3, 5, 20–22), then it may also be that HCR rats would exhibit enhanced depressor and sympathoinhibitory responses compared with LCR rats to other treatments, such as vasopressin V1a receptor blockade in the RVLM, that have been reported to contribute to cardiorespiratory modulation in CIH (24). Other possible mechanisms that could contribute to increased MAP in CIH-treated animals is activation of ANG receptors (5) and reduced NO-mediated inhibition (3) in the PVN and increased oxidative stress and impaired vasodilation (27, 44). Of note, non-PVN-dependent neural control mechanisms contribute to hypertension induced by CIH. These most notably include activation of sympathoexcitatory neurons in the RVLM together with antecedent neurons in the NTS that transmit the arterial chemoreflex (26, 42, 43).

Whereas PVN-injected muscimol reduced MAP in both HCR and LCR rats, it did not significantly change ongoing SSNA in either group. The splanchnic nerve was examined here to explore the sympathetic underpinnings of cardiovascular and metabolic disturbances in LCR rats compared with HCR rats. However, since previous studies in humans and rats exposed to CIH have suggested that increased SNA is responsible for the increased MAP (8, 45), and because the PVN is important for the sympathetic regulation of ABP in CIH-exposed rats (21), we expected PVN-injected muscimol to
decrease SSNA. Failure to observe this suggests that SSNA does not contribute to the maintenance of MAP in HCR and LCR rats exposed to CIH. Additional studies are needed to determine the mechanisms that account for the depressor response to PVN-injected muscimol. Likely possibilities include decreases of SNA to nonplanchnic vascular beds, inhibition of putitary vasopressin release, or both.

In summary, our data suggest that rat strains exhibiting different body weights with intrinsic differences in aerobic capacity and O2 utilization efficiency have nearly identical hypertensive responses to CIH. However, the central autonomic control mechanisms that maintain hypertension differ between HCR and LCR strains in that hypertension in HCR rats is more reliant on ongoing PVN neuronal activity. Thus, whereas phenotypic differences across strains appear to be associated with different adaptive responses to CIH, the overall impact of CIH on arterial pressure is strain invariant.

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The LCR and HCR model can be made available for collaborative study (contact: brittons@umich.edu or lgkoch@umich.edu).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


