9-1-2002

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Matthew Hodges
Medical College of Wisconsin

Hubert Forster
Medical College of Wisconsin

Paula Papanek
Marquette University, paula.papanek@marquette.edu

Melinda R. Dwinell
Medical College of Wisconsin

Genevieve E. Hogan
Medical College of Wisconsin

Accepted version. *Journal of Applied Physiology*, Vol. 93, No. 3 (September 2002): 974-983. DOI.

Journal of Applied Physiology, Vol. 93, No. 3 (2002): 974-983. DOI.

Ventilatory phenotypes among four strains of adult rats

Matthew R. Hodges
Department of Physiology, Medical College of Wisconsin
Hubert V. Forster
Department of Physiology, Medical College of Wisconsin
Zablocki Veterans Affairs Medical Center
Paula E. Papanek
Department of Physical Therapy, Marquette University, Milwaukee, WI
Melinda R. Dwinell
Department of Physiology, Medical College of Wisconsin

Genevieve E. Hogan
Department of Physiology, Medical College of Wisconsin

Abstract

Our purpose in this study was to identify different ventilatory phenotypes among four different strains of rats. We examined 114 rats from three in-house, inbred strains and one outbred strain: Brown Norway (BN;*n* = 26), Dahl salt-sensitive (*n* = 24), Fawn-hooded Hypertensive (FHH: *n* = 27), and outbred Sprague-Dawley rats (SD; *n* = 37). We measured eupneic (room air) breathing and the ventilatory responses to hypoxia (12% O_2-88% N_2), hypercapnia (7% CO_2), and two levels of submaximal exercise. Primary strain differences were between BN and the other strains. BN rats had a relatively attenuated ventilatory response to CO_2 ($P < 0.001$), an accentuated ventilatory response to exercise ($P < 0.05$), and an accentuated ventilatory roll-off during hypoxia ($P < 0.05$). Ventilation during hypoxia was lower than other strains, but hyperventilation during hypoxia was equal to the other strains ($P > 0.05$), indicating that the metabolic rate during hypoxia decreased more in BN rats than in other strains. Another strain difference was in the frequency and timing components of augmented breaths, where FHH rats frequently differed from the other strains, and the BN rats had the longest expiratory time of the augmented breaths (probably secondary to the blunted CO_2 sensitivity). These strain differences not only provide insight into physiological mechanisms but also indicate traits (such as CO_2 sensitivity) that are genetically regulated. Finally, the data establish a foundation for physiological genomic studies aimed at elucidating the genetics of these ventilatory control mechanisms.

Variability in physiological phenotypes, including variability in baseline ventilation as well as ventilatory responses to hypoxia and hypercapnia, is thought to be the product of two main influences: genetic and environmental. It is difficult to decipher each of these components' contribution to the overall physiological variability, particularly in the human population. The contribution of genetic influences to ventilatory responses to hypoxia and/or hypercapnia in humans has been studied by comparing monozygotic and dizygotic twins ([4], [14]). However, conclusions from these experiments have been somewhat equivocal. In contrast, by studying inbred animal models, where both genes and environment can be controlled by design, the relative contribution of each can be elucidated or at least estimated. There have been several studies that have aimed to decipher the possible role of genetic determinants in the control of breathing in both inbred mouse and rat models, both of which have furthered our understanding of the genetic determinants of the control of breathing ([1], [10], [11], [18], [27-35]). Given that human, mouse, and rat genome sequences are complete (or near complete), the power of studying inbred models lies in attaching the physiology to the genome and allowing for more specific hypotheses in studying the control of breathing in humans.

In 1997, Strohl et al. [27] described differences between inbred rat strains, as well as differences between male and female rats during eupnea, hyperoxia, hypoxia, and hyperoxic hypercapnia. In particular, compared with the Koletsky and Brown Norway (BN) rats, both Zucker and Sprague-Dawley (SD) rats exhibited a greater increase in minute ventilation ($\dot{V}e$) in response to hypercapnia, which was deemed to be independent of effects of sex and weight. A conclusion from this study was that the strain of rat, or genetic background, has a major influence on ventilation in response to acute exposure to hypoxia and hypercapnia.

Similar findings of genetic determinants in the control of breathing have been identified in inbred mouse strains [18,29-35]. Tankersley et al. [31, 33, 35] described phenotypic differences among eight inbred mouse strains and concluded that genetic determinants govern interstrain variation in the magnitude and pattern of breathing during hypoxia and hypercapnia. This study launched a series of experiments aimed to further the understanding of genetic influences in many aspects of ventilation, including the nature of inheritance of baseline breathing patterns, lung mechanics, and the acute hypoxic ventilatory response. Utilizing the F2 intercross and recombinant inbred strain approaches, Tankersley and colleagues [29, 30] have been able to link eupneic inspiratory timing to mouse chromosome 3, as well asV˙e, tidal volume (Vt), and mean inspiratory flow in response to hypoxia to chromosome 9. These studies provide evidence for a specific genetic influence in ventilatory control mechanisms, as well as the existence of phenotypic differences among different inbred and outbred rodent strains.

There is great controversy surrounding the origin of the exercise hyperpnea, and investigation of this phenomenon has recently come to a virtual standstill [8]. In a recent review, Forster [8] states “the mechanism of the exercise hyperpnea remains controversial because investigators have yet to devise an ideal preparation to study the phenomenon.” Forster [8] proposed studies utilizing phenotypic differences between inbred rodent strains and molecular genetics techniques to determine the genes involved in the mechanism. However, the utility of this approach would be enhanced with documented differences in the ventilatory response to exercise among inbred animals, which to our knowledge has never been studied.

Elucidating phenotypic differences among different rat strains may not only be valuable for determining the genetic basis of variation in physiological behaviors but also directly provide insight into the fundamental physiological mechanisms in the control of breathing. Therefore, the objective of this study was to determine whether there are phenotypic differences in eupneic ventilation and in the ventilatory response to hypoxia, hypercapnia, and exercise among inbred [BN, Dahl salt-sensitive (SS), and Fawn-hooded hypertensive (FHH)] and outbred (SD) rat strains. We chose a comprehensive approach to determine whether phenotypic differences were specific to a particular stimulus or a general characteristic of ventilatory control. To minimize the influence of environmental factors, we chose to study these three inbred strains because they have been maintained in our animal facility for many generations. In light of the findings of Strohl et al. [28], we hypothesize that the inbred BN strain from our Medical College of Wisconsin colony (BN/MCW) will also exhibit a blunted response to hypercapnia relative to the outbred SD rats. Furthermore, because outbred (Wistar) rats have been shown to hyperventilate in response to exercise [9], we hypothesize that BN, SS, FHH, and SD rats will also hyperventilate in response to submaximal exercise.

METHODS

Strains.

A total of 114 adult (8–10 wk of age) male and female rats from three in-house, inbred rat strains and one outbred strain were studied: Dahl SS, BN, and FHH, and SD strains. The origins of both the SS and BN rats have been published [5]. FHH rats were originally part of a colony of inbred rats from Erasmus University in Rotterdam (Rotterdam, The Netherlands). Complete homozygosity in each of these inbred strains has been verified (H. Jacob, unpublished data). BN, SS, and FHH rats are well-

established models that are routinely utilized in cardiovascular and renal studies, particularly due to their normotensive and hypertensive phenotypes, respectively. We also chose to study an outbred strain of SD rats commercially purchased from Harlan Sprague Dawley (Indianapolis, IN). Being an outbred model, the SD's genetic composition is assumed to be random heterozygosity/homozygosity and provide a comparative standard model for our inbred strains.

All inbred strains are housed and produced at the Medical College of Wisconsin in the Animal Resource Center Transgenic Barrier facility before and during the experimental protocol. Commercially purchased SD rats were housed for 1 wk before testing. All animals were under supervision of the Animal Resource Center staff and provided food and water ad libitum. All protocols were reviewed and approved by the Medical College of Wisconsin Animal Care Committee.

Surgical procedure.

Femoral arterial catheters were chronically implanted in rats for direct measurement of heart rate (HR) and blood pressure and for sampling arterial blood for determination of blood gases and pH. Anesthesia was induced with an injection of xylazine (2 mg/kg im) and ketamine (30 mg/kg im). The indwelling femoral catheter was fixed, tunneled subcutaneously, and externalized near the back of the head. The externalized portion of the catheter was housed in a spring secured to the skin to protect the catheter. The catheterized animals were allowed a minimum of 1 wk to recover after surgery before initiation of experimentation. Femoral catheters were flushed every 1–2 days during the recovery week to ensure patency and proper flow maintenance.

Experimental design: eupneic ventilation and response to hypoxia and hypercapnia.

Ventilatory responses to hypoxia and hypercapnia were determined by using standard plethysmographic techniques in a custom-made, 10-liter Plexiglas plethysmograph [7]. The animals were acclimated to the plethysmograph for 20 min/day for several days before the experimental protocol. On the day of experimentation, the rats acclimatized for ~10 min before data collection with the chamber open to room air. The chamber was closed, and control data were collected for 5 min. Immediately after the control period, gas (4.1 liters 100% N_2 or 0.650 liter 100% CO_2) was injected via input ports and circulated with an internal electric fan, and experimental data were collected for 10 min. Arterial blood samples (0.3 ml) were drawn for the hypoxia protocol during *minutes 3–4*

and *7–8* for the control and hypoxia periods, respectively. Blood samples were drawn through a catheter connected to the femoral line and externalized via custom-made screw-cap ports in the plethysmograph to minimize the disturbance of the animal. Air temperature inside the plethysmograph (23.3 ± 0.2°C) and the relative humidity (63.4 ± 1.6%) were monitored by using a calibrated Omega RX-93 temperature and telative humidity probe. Gases were administered and sampled via an exhaust port, and measured with calibrated O_2 and CO_2 gas analyzers (Applied Electrochemistry models S-3A/I and CD-3A, respectively). Rectal temperatures were obtained before and after experimentation with a calibrated thermocouple probe. Ventilation was monitored with a SENSYM model SCX-E1 pressure transducer, which was calibrated by using a pressure wave created with a 1-ml syringe (volume of 0.3 ml at a frequency of 2 Hz) when the animal is in the chamber at the beginning of the control period and during the final minute of the experimental exposure series.

Experimental design: ventilatory response to exercise.

Rats were trained daily for exercise on a commercially available four-lane rodent treadmill (Columbus Instruments) at speeds of 0.8 and 1.8 m/min at 5% grade for 5–10 min for 1 wk before catheter instrumentation. On the day of experimentation, rats were placed on the treadmill and allowed to rest quietly for 10 min, during which time arterial blood pressure and HR were monitored. Arterial blood samples (0.3 ml) were drawn from the indwelling femoral catheter at the end of the resting period and during the final minute of each level of exercise. As in past studies ([20], [21]), arterial Pco_2 (Pa_{CO2}) was used to assess the ventilatory response to exercise. Arterial blood gases [Pa_{CO2}, arterial Po_2(Pa_{O2})], arterial pH, and percent O_2 saturation values were obtained by using a Chiron Rapidlab model 840 blood-gas analyzer (Bayer). HR and blood pressure were monitored continually except during acquisition of arterial blood samples.

Data acquisition and statistical analysis.

V˙e, Vt, breathing frequency (f), inspiratory and expiratory time (Ti and Te, respectively), mean arterial blood pressure (MAP), and HR were obtained by using data-acquisition software (CODAS) at a sampling rate of 100 samples/s. The plethysmograph data were segmented and sorted into bins (control and

minutes 0–3 and *7–10* of hypoxia and hypercapnia). Each segment of data used in this analysis was between 30 and 60 s of continuous breathing and determined not to be sniffs or sighs to ensure accurate mean values. Raw data segments were analyzed by using a software program (Windaq Playback) designed to detect peaks and valleys, and timing and integration calculations for ventilation, MAP, and HR. Vt was calculated (and calibrated) by using the methods of Drorbaugh and Fenn [6], and multiplied by frequency to obtain$\dot{V}$e. Use of this method factors individual body temperatures into the Vt calculations.

Spontaneous augmented breaths (ABs) were observed in both sexes in all strains during all resting conditions. ABs were biphasic in nature and defined as a breath that, during the inspiratory phase, exhibits a typical slow rate of rise to a normal Vt, followed by a rapid rate of rise to a peak volume of at least twice that of eupneic Vt. The expiratory phase was a slowly decrementing pattern, followed by an apnea before the reestablishment of subsequent breaths. The breaths studied were the 13 breaths before the AB and the AB. Overall frequency of the AB, Ti, and Te of the AB [also termed postsigh apnea (PSA)], as well as anticipation of the AB were analyzed. The total cycle time of each of the three breaths before the AB (N-3, N-2, N-1, consecutively) were normalized to the average of the 10 breaths before the N-3 breath. Therefore, cycle times for each breath were expressed as a percentage of the control cycle time. Significant lengthening of the cycle time in the N-1 breath (compared with the N-3 and/or N-2 breaths) was indicative of anticipation of the AB. All data on ABs are average values from a minimum of three ABs for each animal in each condition, which were then averaged to obtain mean strain values.

Within-strain variation between experimental time points and male vs. female comparisons were assessed with an unpaired *t*-test. Between-strain variation was assessed with a one-way ANOVA followed with a Bonferroni post hoc test. All statistical analyses were limited to a 95% confidence interval to test for significant differences between groups. Equality of variance between strains in physiological variables was assessed by Levene's test [13].

RESULTS

Eupneic ventilation: effect of strain and sex.

Eupneic Vt and V˙e were normalized to body weight due to significant strain variation (Table1). There were no strain differences among all male rats in eupneic V˙e, f, Vt, and Te, but female rats did exhibit significant ($P < 0.05$) interstrain variability (see Table 1 for details). Arterial blood-gas and acid-base data during eupnea for male and female rats were pooled due to the lack of sufficient data on female rats. With one exception, there were no differences ($P > 0.05$) among all strains in pooled Pa_{CO2}, Pa_{O2}, and arterial pH (Table 1). As expected, SS and FHH rats exhibited higher MAP ($P \leq 0.003$) compared with both BN and SD rats, and MAP was greater ($P = 0.048$) in SD rats compared with BN rats. Despite large interstrain variation in MAP, there were no differences in the resting HRs among all strains studied.

Table 1. Characteristics of inbred rat strains breathing room air

Strain	Sex	BN 18 M, 8 F	SS 16 M, 8 F	FHH 17 M, 10 F	SD 21 M, 16 F	Strain Difference[†]
Weight, g	M	265.8 ± 9.7*	310.0 ± 14.9*	308.9 ± 4.9*	336.6 ± 4.2*	SS, SD>BN
	F	150.9 ± 6.9	213.6 ± 6.8	218.6 ± 2.8	218.6 ± 3.6	ALL>BN
Temp, °C	M	37.23 ± 0.22	37.80 ± 0.11	37.27 ± 0.16	37.23 ± 0.19	NS
	F	38.23 ± 0.25*	38.19 ± 0.10	37.73 ± 0.15	37.05 ± 0.19	BN, SS>SD
V˙e, ml · min^{-1} · 100 g^{-1}	M	11.33 ± 0.45	10.954 ± 0.508	12.517 ± 0.604	11.357 ± 0.442	NS
	F	16.11 ± 0.73*	13.316 ± 0.760*	15.986 ± 1.267*	12.585 ± 0.576	BN, FHH>SD
f, breaths/min	M	93.1 ± 2.3	88.4 ± 2.6	96.0 ± 2.9	95.9 ± 2.8*	NS
	F	85.1 ± 3.9	84.3 ± 3.5	102.6 ± 5.5	81.0 ± 2.8	FHH>ALL
Vt, ml · $breath^{-1}$ · 100 g^{-1}	M	0.123 ± 0.005	0.124 ± 0.005	0.132 ± 0.006	0.122 ± 0.005	NS
	F	0.193 ± 0.010*	0.159 ± 0.007*	0.156 ± 0.009*	0.159 ± 0.007*	BN>FHH
Ti, s	M	0.250 ± 0.010	0.253 ± 0.012	0.246 ± 0.011	0.209 ± 0.007	BN, SS>SD
	F	0.245 ± 0.014	0.260 ± 0.008	0.257 ± 0.014	0.244 ± 0.007*	NS
Te, s	M	0.410 ± 0.014	0.450 ± 0.020	0.376 ± 0.016	0.441 ± 0.020	NS

Strain	Sex	BN 18 M, 8 F	SS 16 M, 8 F	FHH 17 M, 10 F	SD 21 M, 16 F	Strain Difference[†]
	F	0.484 ± 0.032[*]	0.471 ± 0.027	0.396 ± 0.019	0.524 ± 0.026[*]	SD>FHH
Pa_{CO2}, Torr		34.26 ± 0.68	35.60 ± 0.88	36.96 ± 0.78	35.51 ± 0.59	NS
Pa_{O2}, Torr		99.65 ± 2.94	103.96 ± 1.50	95.89 ± 2.04	102.82 ± 1.90	SS>FHH
pH		7.456 ± 0.010	7.441 ± 0.004	7.439 ± 0.006	7.434 ± 0.005	NS
MAP		105.6 ± 2.5	127.1 ± 2.2	128.5 ± 2.1	115.0 ± 2.5	SS, FHH>BN, SD SD>BN
HR		415.3 ± 14.0	414.5 ± 10.7	422.5 ± 12.8	424.4 ± 10.0	NS

Values are means ± SE for weight, rectal temperature (Temp), minute ventilation (V˙e), breathing frequency (f), tidal volume (Vt), inspiratory time (Ti), expiratory time (Te), Pa_{CO2}, Pa_{O2}, arterial pH, mean arterial pressure (MAP), and heart rate (HR) for Brown Norway (BN), salt-sensitive (SS), Fawn-hooded hypertensive (FHH), and Sprague-Dawley (SD) male (M) and female (F) rats during resting conditions. ALL, all strains; NS, not significant.

[*]M/F comparison,

[†]$P < 0.05$. Between-strain differences, $P < 0.05$.

Ventilatory response to hypoxia.

In light of the differences in body weight, the ventilatory responses to hypoxia (and hypercapnia) were expressed as percentage of control (eupnea) to normalize data between strains. Among the hypoxia and hypercapnia data, there were only seven significant differences detected out of 48 total comparisons (14.6%) between males and females in all strains for all ventilatory parameters. Therefore, data were pooled to obtain mean values for each strain.

During *minutes 0–3*, V˙e and f were increased ($P < 0.001$) from control in all strains, and the increase was greater in SS rats than in BN rats ($P \leq 0.038$; Fig. 1, Table 2). During *minutes 7–10* of hypoxia, V˙e and f were increased ($P < 0.001$) from control in SS, FHH, and SD rats ($P < 0.001$), whereas f ($P = 0.012$) but not V˙e increased in BN rats ($P > 0.05$). Only BN rats decreased V˙e and Vtsignificantly ($P \leq 0.005$) from *minutes 0–3* to *7–10* of hypoxia, which indicates a greater hypoxic ventilatory roll-off in BN rats. All four strains exhibited little interstrain variation in Vt, arterial blood gases, or arterial pH, or in the change in Pa_{O2} (Table 2) and Pa_{CO2} (Fig. 2) between normoxia and hypoxia, indicative of equivalent stimulus levels and responses. There was no change in HR, and FHH rats were the only rats to significantly reduce MAP during hypoxia (−9.6%;$P = 0.028$).

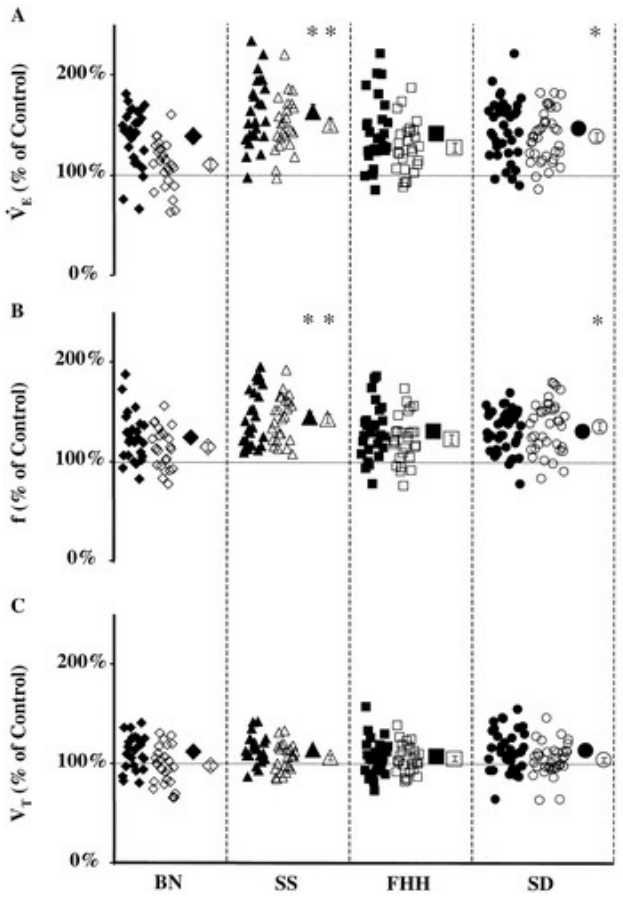

Fig. 1. Ventilatory response to hypoxia in 4 strains of rats. Individual (small symbols) and mean (large symbols) ± SE data for minute ventilation (V˙e; *A*), breathing frequency (f; *B*) and tidal volume (Vt;*C*) were expressed as a percentage of control for 2 time points [*minutes 0–3* (solid symbols), *minutes 7–10* (open symbols)] during hypoxic challenge. Note that salt-sensitive rats (SS; ▲, ▵) increasedV˙e and f greater than Brown Norway rats (BN; ⧫, ◊) at both time points (* $P < 0.05$), but no significant differences were found in Vt among all strains during hypoxia. Sprague-Dawley rats (SD; ●, ○) increasedV˙e and f greater than BN rats during *minutes 7–10* of hypoxia (* $P < 0.05$). FHH, Fawn-hooded hypertensive rats (■, □).

Table 2. Ventilation during hypoxia (12% O_2-0.03% CO_2-balance N_2)

Strain	Time, min	BN	SS	FHH	SD	Strain Difference*
V˙e, ml · min^{-1} · 100 g^{-1}	*0–3*	17.61 ± 1.25	19.37 ± 0.94	21.08 ± 0.96	18.43 ± 1.10	NS
	7–10	14.20 ± 1.03	17.98 ± 0.93	19.37 ± 1.02	17.20 ± 0.86	FHH>BN
f, breaths/min	*0–3*	110.8 ± 3.6	124.1 ± 4.6	137.3 ± 4.9	112.9 ± 4.0	FHH>BN, SD
	7–10	102.4 ± 3.4	122.6 ± 3.2	129.6 ± 4.4	116.7 ± 4.2	SS, FHH>BN

Table 2. Ventilation during hypoxia (12% O_2-0.03% CO_2-balance N_2)

Strain	Time, min	BN	SS	FHH	SD	Strain Difference*
Vt, ml · breath^{-1} · 100 g^{-1}	*0–3*	0.157 ± 0.008	0.158 ± 0.007	0.154 ± 0.008	0.164 ± 0.009	NS
	7–10	0.138 ± 0.009	0.147 ± 0.006	0.150 ± 0.008	0.151 ± 0.008	NS
Ti, s	*0–3*	0.229 ± 0.007	0.173 ± 0.006	0.174 ± 0.006	0.194 ± 0.006	BN>ALL
	7–10	0.252 ± 0.007	0.182 ± 0.006	0.190 ± 0.006	0.193 ± 0.006	BN>ALL
Te, s	*0–3*	0.333 ± 0.017	0.321 ± 0.012	0.272 ± 0.010	0.365 ± 0.016	SD, BN<FHH
	7–10	0.355 ± 0.020	0.315 ± 0.011	0.291 ± 0.016	0.346 ± 0.017	NS
Pa_{CO2}, Torr		27.17 ± 1.31	28.72 ± 0.83	29.01 ± 0.55	27.64 ± 0.51	NS
Pa_{O2}, Torr		51.14 ± 2.48	54.27 ± 1.19	47.99 ± 1.06	52.06 ± 1.11	SS>FHH
pH		7.502 ± 0.014	7.496 ± 0.006	7.489 ± 0.013	7.506 ± 0.005	NS

Values are means ± SE for V˙e, f, Vt, Ti, Te, Pa_{CO2}, Pa_{O2}, and arterial pH for BN, SS, FHH, and SD rats while inspiring 12% O_2.
*P values for between strain difference of <0.05.

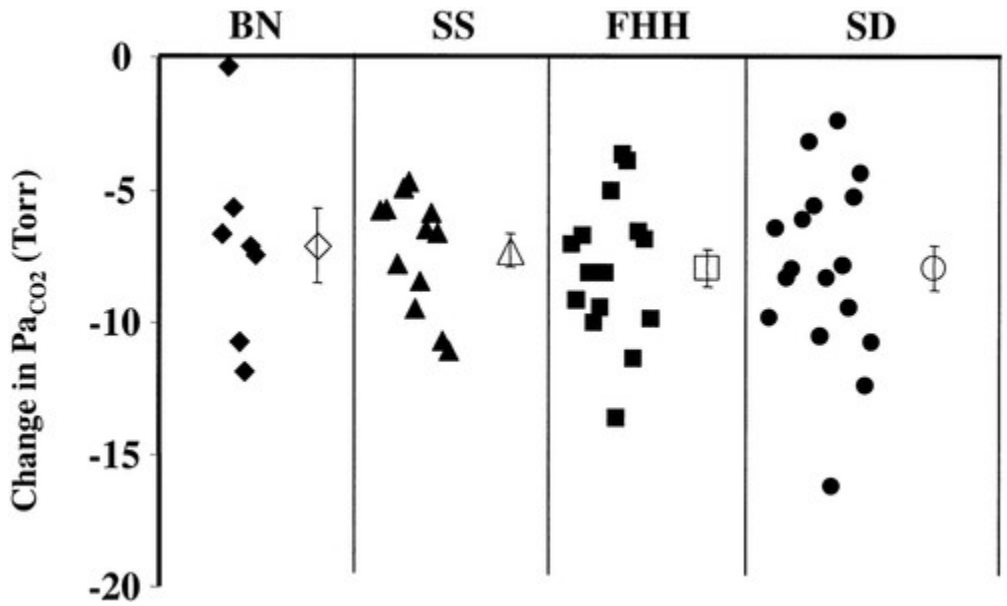

Fig. 2. Changes from eupnea in arterial Pco_2 (Pa_{CO2}) during hypoxia. Individual (solid symbols) and mean (open symbols) ± SE data for the change in arterial Pa_{CO2} from eupnea during hypoxia are plotted for BN (⧫, ◊), SS (▲, ▵), FHH (■, □), and SD rats (●, ○). All strains decreased Pa_{CO2}significantly from control ($P < 0.05$) during hypoxia, but no between-strain differences were detected ($P > 0.05$).

Response to hypercapnia.

V˙e, f, and Vt increased from eupnea in all strains at all time points during hypercapnia ($P \leq 0.01$; Table 3). SS, FHH, and SD rats increased V˙e and f more than BN rats during hypercapnia ($P < 0.001$; Fig.3, *A–C*), and the increase in Vt (from control) was greater in SS than in all other strains during *minutes 7–10* ($P \leq 0.004$). Additionally, BN rats exhibited the longest Ti($P < 0.001$) and Te ($P \leq 0.012$) throughout hypercapnia (Table 3). There were no significant changes in MAP, and only BN rats decreased HR significantly from control (31.1% reduction; $P = 0.001$).

Table 3. Ventilation during hypercapnia (7% CO_2-93% O_2)

Strain	Time, min	BN	SS	FHH	SD	Strain Difference[3-150]
V˙e, ml · min^{-1} · 100 g^{-1}	*0–3*	22.08 ± 2.03	34.28 ± 1.89	28.90 ± 1.95	31.40 ± 1.46	SS, SD>BN
	7–10	18.78 ± 1.60	35.16 ± 1.84	27.17 ± 1.59	29.72 ± 1.46	SS, SD>BN, SS>FHH
f, breaths/min	*0–3*	108.6 ± 5.0	150.7 ± 5.3	149.8 ± 4.8	153.6 ± 2.2	ALL>BN
	7–10	103.2 ± 3.8	151.9 ± 4.0	148.4 ± 4.2	155.1 ± 1.9	ALL>BN
Vt, ml · $breath^{-1}$ · 100 g^{-1}	*0–3*	0.199 ± 0.014	0.229 ± 0.013	0.195 ± 0.011	0.206 ± 0.010	NS
	7–10	0.180 ± 0.012	0.232 ± 0.011	0.185 ± 0.009	0.194 ± 0.010	SS>BN, FHH
Ti, s	*0–3*	0.251 ± 0.009	0.191 ± 0.008	0.188 ± 0.005	0.175 ± 0.003	BN>ALL
	7–10	0.274 ± 0.009	0.186 ± 0.006	0.190 ± 0.008	0.177 ± 0.003	BN>ALL
Te, s	*0–3*	0.337 ± 0.023	0.209 ± 0.007	0.221 ± 0.007	0.214 ± 0.005	BN>ALL
	7–10	0.332 ± 0.017	0.215 ± 0.005	0.229 ± 0.009	0.212 ± 0.004	BN>ALL

Values are means ± SE for$V˙_E$, f, V_T, T_I, T_E for BN, SS, FHH, and SD rats while inspiring 7% CO_2.
[F3-150]Between-strain difference, $P < 0.05$.

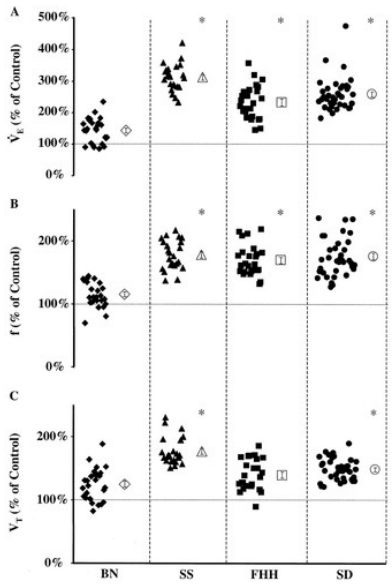

Fig. 3. Ventilatory response to hypercapnia in 4 strains of rats. Individual (solid symbols) and mean (open symbols) ± SE data for V˙e (*A*), f (*B*), and Vt (*C*) expressed as a percentage of control for*minutes 7–10* of hypercapnic challenge. Note that all strains increase V˙e from control (*P*< 0.001), and SS (▲ ▵), FHH (■, □), and SD rats (●, ○) increased V˙e and frequency more than BN rats (■, □) during *minutes 7–10* of hypercapnia (* *P* < 0.001). Also, SS rats increased V˙e and Vt greater than all other strains during *minutes 7–10* of hypercapnia (*P* < 0.001).

Ventilatory response to exercise.

Compared with Pa_{CO2} values obtained while in the plethysmograph, all strains hyperventilated (*P* ≤ 0.02) while at rest on the treadmill except the BN rats (Table4). Relative to rest, BN, FHH, and SD rats exhibited reductions in arterial Pa_{CO2} (*P* ≤ 0.05) during the first level of exercise, and all strains lowered (*P* ≤ 0.018) Pa_{CO2} from resting levels in response to the second level of exercise (Fig. 4). The decrease in arterial Pa_{CO2} in BN rats during the first level of exercise was greater than the reduction seen in SS rats (*P* = 0.013), but there were no differences in the decrease in Pa_{CO2} between strains at the second level of exercise. BN and SS rats did not alter MAP from resting levels; however, FHH and SD rats increased MAP (*P* < 0.05) from resting to the second level of exercise (111.2–129.1 mmHg and 100.4–113.8 mmHg, respectively). All rats increased HR from rest to both levels of exercise (*P* ≤ 0.02, data not shown).

Table 4. Arterial blood gases and pH during two levels of submaximal exercise

Strain	Ex Level	BN	SS	FHH	SD	Strain Difference4-150
Pa_{CO2}, Torr	Rest	32.50 ± 1.23	32.63 ± 1.18	32.85 ± 1.04	31.40 ± 0.60	NS
	1	27.80 ± 0.90	29.17 ± 0.57	28.67 ± 0.56	30.33 ± 0.48	SD>BN
	2	23.83 ± 0.72	26.12 ± 1.18	26.30 ± 0.92	27.16 ± 0.97	NS
Pa_{O2}, Torr	Rest	106.2 ± 4.02	110.6 ± 3.78	117.6 ± 6.18	104.4 ± 4.73	NS

Strain	Ex Level	BN	SS	FHH	SD	Strain Difference[4-150]
	1	106.2 ± 2.74	109.3 ± 3.71	109.2 ± 2.73	114.7 ± 4.99	NS
	2	116.3 ± 2.37	114.7 ± 4.32	125.6 ± 3.04	118.7 ± 3.78	NS
pH	Rest	7.456 ± 0.004	7.469 ± 0.005	7.481 ± 0.011	7.463 ± 0.012	NS
	1	7.458 ± 0.005	7.465 ± 0.011	7.495 ± 0.007	7.462 ± 0.013	SS>SD
	2	7.409 ± 0.009	7.472 ± 0.014	7.472 ± 0.006	7.450 ± 0.011	SS>SD

Values are means ± SE for arterial Po_2 (Pa_{O2}), arterial Po_2 (Pa_{O2}), and arterial pH for BN, SS, FHH, and SD during 2 levels of treadmill running (Ex level).
[F4-150]Between-strain difference, $P < 0.05$.

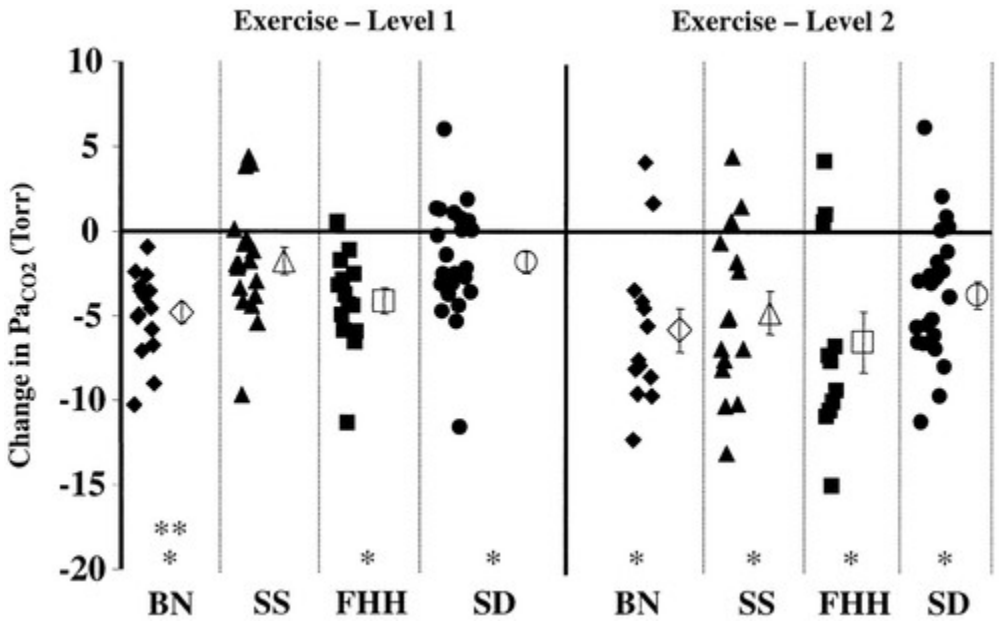

Fig. 4. Ventilatory response to exercise expressed as the change in Pa_{CO2} from resting values to *exercise level 1* (0.8 m/min) or *exercise level 2* (1.8 m/min) for BN (⧫, ◊), SS (▲, ▵), FHH (■, □), and SD rats (●, ○). Individual (solid symbols) and mean (open symbols) ± SE data are shown. Note that BN rats decreased Pa_{CO2} more than SS rats during the first level of exercise (** $P < 0.05$) and that all strains decreased Pa_{CO2} from rest during*level 2* (* $P < 0.05$) but did not differ from one another ($P > 0.05$).

Phenotypic characteristics of ABs.

All rats studied exhibited spontaneous ABs under all conditions (Table5). A total of 11 significant differences out of 92 comparisons (12.0%) between male and female rats was detected, and, therefore, the data for AB characteristics [with the exception of the N-1 duration of respiratory cycle (Ttot) data] were pooled. FHH rats had fewer ($P < 0.05$) ABs during eupnea and hypoxia than all other strains, and all strains increased AB frequency during hypoxia ($P < 0.001$). With the exception of SD rats, all other

strains increased ($P < 0.05$) AB frequency during hypercapnia, although the increase was modest in relation to the increase observed during hypoxia.

Table 5. AB characteristics during eupnea, hypoxia, and hypercapnia

Strain	Condition	BN	SS	FHH	SD	Strain Difference[5-151]
AB frequency, AB/min[5-151]	Eupnea	0.566 ± 0.02	0.558 ± 0.25	0.437 ± 0.03	0.615 ± 0.04	FHH<ALL
	Hypoxia	1.487 ± 0.09[5-150]	1.742 ± 0.08[5-150]	1.024 ± 0.04[5-150]	2.532 ± 0.13[5-150]	SD>ALL, FHH<ALL
	CO_2	0.793 ± 0.06[5-150]	0.567 ± 0.04[5-150]	0.602 ± 0.04[5-150]	0.637 ± 0.03	BN>FHH
AB Ti, s	Eupnea	0.527 ± 0.04	0.486 ± 0.03	0.212 ± 0.02	0.372 ± 0.01	SS, BN>FHH, SD
	Hypoxia	0.472 ± 0.07	0.338 ± 0.01[5-150]	0.204 ± 0.02	0.340 ± 0.01	BN>FHH
	CO_2	0.407 ± 0.02	0.334 ± 0.02[5-150]	0.173 ± 0.02	0.284 ± 0.01[5-150]	BN>ALL, SS, SD>FHH
AB Te, s	Eupnea	2.220 ± 0.15	1.392 ± 0.10	1.264 ± 0.13	1.493 ± 0.11	BN>ALL
	Hypoxia	1.968 ± 0.24	0.976 ± 0.10[5-150]	0.963 ± 0.07[5-150]	1.101 ± 0.14[5-150]	BN>ALL
	CO_2	1.123 ± 0.14[5-150]	0.314 ± 0.02[5-150]	0.553 ± 0.03[5-150]	0.344 ± 0.01[5-150]	BN>ALL

Values are means ± SE for augmented breath (AB) frequency, AB Ti, and AB Te for BN, SS, FHH, and SD during eupnea (room air), hypoxia (12% inspired O_2-88% N_2), and hypercapnia (7% CO_2).
[F5-150] $P < 0.05$ for comparison with eupnea.
[F5-151] P value for between strain difference of <0.05.

During eupneic, hypoxic, and hypercapnic conditions, BN rats had a longer PSA defined as Te of the AB) than all other strains ($P \leq 0.001$; Table 5, Fig.5). The individual data for the PSA measurements are depicted to illustrate the large variation in BN rats, as well as the strain differences during eupnea, hypoxia, and hypercapnia. BN rats also tended to have the longest Ti of the AB (Table 5), although strain differences were not as obvious as seen with the PSA.

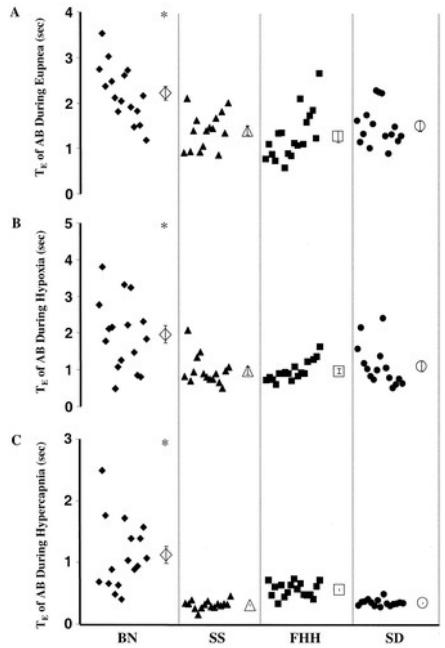

Fig. 5. Individual (solid symbols) and mean (open symbols) ± SE data for expiratory time (Te) of augmented breaths (AB) for BN (⧫, ◊), SS (▲ , ▵), FHH (■, □), and SD rats (●, ○) during eupnea (*A*), hypoxia (*B*), and hypercapnia (*C*). Note that during eupnea, hypoxia, and hypercapnia, BN rats have a longer Te of the AB than all other strains (* $P \leq 0.001$). * Comparison with eupnea, $P < 0.05$.† Between-strain difference, $P < 0.05$.

Although most AB characteristics were similar between male and female rats, we note strain-specific and gender-specific effects in the anticipatory phase of the AB. Anticipation was defined as a lengthened total cycle time in the breath immediately preceding the AB (Ttot of N-1 breath) compared with the average Ttot of 10 control breaths that precede the event. Under all conditions (eupnea, hypoxia, and hypercapnia), male BN and SS rats consistently exhibited an anticipatory phase of the AB (data not shown). In contrast, there was no evidence of anticipation in female BN and SS in and both male and female FHH and SD rats under all conditions.

Equality of variance.

The variance in SD rats was greater ($P < 0.05$) than one or more of the inbred strains studied when comparing eupneicV˙e, f, Vt, and Te. However, there were no differences in variance in nearly 50% of comparisons during eupnea, hypoxia, and hypercapnia.

DISCUSSION

In this study, we report strain-specific ventilatory phenotypes among four rat strains. The data support our specific hypotheses, in that *1*) like other BN rats, the BN/Mcw rats have a blunted ventilatory response to hypercapnia and *2*) rats from all four strains hyperventilated in response to submaximal exercise. These different phenotypes will not only facilitate future studies directed toward the

elucidation of the genetic determinants of ventilatory control but in addition these differences per se have implications regarding ventilatory control mechanisms.

Physiological implications.

In contrast to eupneic and hypoxic ventilatory responses, our data indicate significant differences in response to hypercapnia among these four strains. In particular, SS, FHH, and SD rats exhibited greater increases in V˙e and f than BN rats. Thus the ventilatory response to hypercapnia appears to be severely blunted in BN rats. These observations are similar to data reported by Strohl et al. [28], where SD rats exhibited greater increases Vt and V˙e than BN rats. It is, however, important to note that the BN rats Strohl et al. studied were obtained from a commercial colony at Harlan Sprague Dawley, whereas the BN rats we have studied were obtained from the in-house colony maintained at the Medical College of Wisconsin. It was, therefore, necessary to establish the phenotypes of our specific strain of BN rats. There was minimal or no overlap in V˙e and f responses to hypercapnia between BN and the other strains of rats (Fig.3); thus it seems that CO_2 sensitivity is genetically regulated. Moreover, because BN rats did not show a blunted response to hypoxia, exercise, or a lower eupneic breathing, this deficit is specific to a CO_2-H^+ sensory and/or processing mechanism and not a result of secondary effects of abnormal breathing mechanics or strain differences in respiratory rhythm or pattern generation.

Each of the rat strains (both inbred and outbred) studied exhibited a significant hyperventilation in response to the second level of submaximal exercise. Specific strain effects in exercise-induced hyperventilation were also apparent, as BN rats decrease Pa_{CO2} more than SS rats during the first level of exercise and exhibit the greatest change in Pa_{CO2} from rest at the second level of exercise (although this change is not significantly different from other strains). The apparent enhanced hyperventilation of BN rats during exercise may indicate a genetic influence in the exercise stimulus for breathing. The finding that enhanced hyperventilation coupled with a relatively low responsiveness to elevated inspired CO_2 is consistent with the concept that the exercise hyperpnea is not mediated by a CO_2-related mechanism. In fact, the hyperventilation observed in BN rats is similar to data reported from carotid body-denervated (CBD) goats, ponies, and dogs where hyperventilation in response to exercise is accentuated after CBD ([2], [7], [21],[22]). This observation raises the possibility that BN rats have deficient chemoreceptive properties at the level of the carotid body. However, hyperventilation during hypoxia in BN rats is not different from the other strains in our study (see below). Thus, if indeed there is abnormal carotid chemoreception in BN rats, it probably is in CO_2-H^+ sensing. It is then relevant that CBD transiently (goats, rats) or permanently (dogs) attenuates CO_2 sensitivity by nearly 60% ([21], [22], [24]). It is possible, although speculative, that the attenuated CO_2 sensitivity (after CBD and in BN rats) results in reduced blunting of a hyperventilatory exercise drive that accounts for the enhanced reduction in Pa_{CO2} during exercise. In other words, the enhanced response to exercise in BN rats may not reflect a genetic difference in the mechanism(s) of the exercise hyperpnea.

Differential responses to hypoxia are influenced by heredity (genetic determinants) in both human and animal studies ([4], [10-12],[14], [19], [27-29], [31], [32]). Among these, Tankersley et al. [32] reported phenotypic differences in the response to acute hypercapnia under normoxia and hypoxic conditions in eight inbred mouse strains. Specific strains exhibited significant differences from other strains in their response to

acute hypoxia and were classified hypoxic high and low responders. A/J mice, or the hypoxic low responders, exhibited an increase in $\dot{V}e$ that was not significant from the hypercapnic normoxic control. Tankersley et al. also reported that $\dot{V}e$ significantly increased with hypercapnic hypoxia (relative to room-air exposure) and that the increase in $\dot{V}e$ was primarily dependent on an increase in f rather than an increase in Vt. Our data are consistent with these observations, where all strains increased$\dot{V}e$ significantly from control during *minutes 0–3* of hypoxia, where f increased significantly but Vt was not different from control. Although all four strains tended to show a decrease in $\dot{V}e$ and Vt over time, BN rats are the only strain that significantly decreased $\dot{V}e$ and Vt from the initial to the final time period, exhibiting a significant ventilatory roll-off. It appears that the data suggest that BN rats have a gene or set of genes that confer a greater hypoxic brain depression.

However, in contrast to the strain differences in the breathing response during hypoxia, analysis of the blood-gas data showed no significant strain differences in eupneic $Pa_{CO2}$and the decrease in Pa_{CO2} and Pa_{O2} during hypoxia. This observation leads us to consider that strain-dependent changes in metabolic rate may govern this disparity. Evidence for differences in metabolic rate during hypoxia have been noted in previous studies that indicate the ratio of ventilation to metabolic rate (flow-to-oxygen consumption ratio) was not significantly different between SD and BN rats when inspiring either 8 or 10% O_2 [(3)]. They also noted mean percent decreases in $\dot{V}o_2$ tended to be greater in BN rats than in SD rats, although only significantly different with 8% inspired O_2. We believe our data in the BN rat of reduced hyperpnea but equal hyperventilation during hypoxia also indicate that the metabolic rate during hypoxia decreased more in BN than in other rats. In other words, the effect of hypoxia per se on ventilation does not differ among the strains, but hypoxia has a differential effect on metabolic rate, which affects ventilation during hypoxia. Furthermore, a common mechanism between the significant ventilatory roll-off and the apparent decrease in$\dot{V}o_2$ in the BN rats is likely, as the two are interrelated. However, it is not possible from the present data to ascertain whether one is primary to these responses. Whatever the mechanism, it most likely is unrelated to CO_2 sensitivity because a low CO_2 sensitivity would confer a reduced ventilatory roll-off during hypoxia.

Despite the wide range of MAPs observed in these hypertensive and normotensive rat strains, we found no differences in$\dot{V}e$, f, or Vt among all male rats during eupnea. Pooled data from male and female rats also show no differences in Pa_{CO2}, arterial pH, and, with one exception, Pa_{O2} (SS > FHH). In light of these findings, our data indicate that there is no discernable difference in the eupneic control of breathing among male BN, SS, FHH, and SD rats. However, this observation may be unique to male rats in our study, as we observed strain differences in eupneic $\dot{V}e$, f, and Vt in our female rats. In a similar investigation, Strohl et al. [(28)] reported significant effects of both strain and sex in eupneic ventilatory phenotypes among SD, BN, Zucker, and Koletsky rats. This group found significant differences in Vt and f but did not find differences between the strains in $\dot{V}e$. SD and Koletsky rats were reported to exhibit a deeper and slower breathing pattern than BN and Zucker animals. In the same report, they also found a specific effect of sex on eupneic f but no differences in Vt or $\dot{V}e$. Herein, we also report significant effects of sex and strain on eupneic parameters but find the strain effects to be unique to female rats. In addition, our data also do not support the slower, deeper breathing pattern observed in SD rats compared with BN rats, as we find no significant differences in Vt or f between SD and BN rats.

The generation of different respiratory rhythms or patterns, such as eupneic, augmented, and gasplike breaths, has recently been the focus of much attention and debate, primarily because of controversies regarding the brain stem site and mechanisms of respiratory rhythm and pattern generation ([17], [25], [26]). Although the physiological implications of the differences in AB phenotypes we observed remain speculative, elucidating the genetic basis of the differences should provide insight into these speculations and controversies. We observed ABs in all rats and under all conditions. During eupnea (normoxia), we observed AB frequency to be 0.57 ± 0.02, 0.56 ± 0.02, 0.44 ± 0.03, and 0.62 ± 0.04 AB/min for BN, SS, FHH, and SD rats, respectively. Leiskie et al. [17] reported a similar sigh (AB) frequency in neonatal rat medullary slice preparations to be $1.10 \times 10^{-2} \pm 1.18 \times 10^{-3}$ Hz, which calculates to 0.66 ± 0.07 AB/min, strikingly similar to the values we report in our adult, intact, unanesthetized SD rats. In addition, they reported that, on introduction of anoxia, AB frequency increased 356.9 ± 57.0%, similar to the increases in frequency of ABs we observed in SD rats (412 ± 27%) during hypoxia. Although we found significant increases in AB frequency in three of the four strains during hypercapnia, the increase was modest compared with those observed during hypoxia. Additionally, the PSA was significantly longer under all conditions in the CO_2-insensitive BN rats compared with all other strains. Therefore, PSA appears to be largely independent of both frequency of occurrence of the AB and the acute hypoxic condition. However, specific components of AB timing appear to be related to relative sensitivity to CO_2.

Genetic implications.

The characterization of the SD rats was originally included to compare and contrast the variance in an outbred strain with the other inbred strains. We had anticipated that the SD rats would exhibit greater within-strain variation than other strains due to assumed genetic heterogeneity. Indeed, variance in eupneic breathing of the SD population was greater than that of one or more of the inbred strains. However, there were no differences in the variance in nearly 50% of all comparisons, suggesting that although the SD rats are assumed to be genetically heterogeneous, there may be one or more factors that influence variation in these rat strains. A possible explanation of why variance in SD rats was not consistently greater than in the inbred strains is that all strains studied possess common alleles that govern the variability in these respiratory phenotypes. Another and more probable explanation for this observation may be that the outbred SD rats may not be as “outbred” as one may think; that is to say that although these rats are randomly bred within a commercial colony, there may be a relatively limited number of alleles in a given gene pool specific to the genetic determinants of respiratory control. Past studies have alluded to this postulate by demonstrating that two different lines of SD rats obtained from different vendors differed in baseline ventilation and ventilatory responses to hypoxia [19]. Additionally, it has been shown in other physiological studies that inbred rat strains exhibit significant variation in quantitative traits. Although these rats are homozygous at all loci, it remains to be explained why genetic “clones” would exhibit variation given relatively equivalent environmental influences.

Inbred rats strains have been shown to be an extremely useful tool in elucidation of genetic determinants of specific physiological control mechanisms by exploiting specific strategies, such as the generation of F2 intercross progeny (F2I), recombinant inbred (RI) strains, and consomic rat strains. With the use of both F2I and RI strains, specific ventilatory phenotypes have been assigned to genomic regions in mice ([29], [30]). Tankersley and colleagues [30] were able to establish 100% concordance

between differential inspiratory timing and genetic markers on mouse*chromosome 3* by utilizing rats from RI lines derived from C3H/HeJ and C57BL/6J progenitors. Additionally, by generating an F2I from the same inbred strains, a quantitative trait locus was identified by linkage analysis for differential Ti and microsatellite markers on *chromosome 3*. Other ventilatory phenotypes, including V˙e, Vt, and Vt/Ti in response to hypoxia, have been linked to a quantitative trait locus on mouse *chromosome 9* by using the same F2I approach.

Alternatively, with the use of marker-assisted selection, consomic rat strains are developed through selective substitution or introgression of an entire chromosome from one inbred strain into the background of the recipient strain. The inheritance of the most robust strain differences (typically where means are different by >2 SD) that are attributed to genetic influences can be explored with chromosomal substitution. Specifically, V˙e and frequency response to hypercapnia, as well as the Te of the AB (PSA) during hypercapnia, not only show little or no overlap in the individual data but also exhibit means that differ by >2 SD. As a result, allelic variants of these quantitative traits after chromosomal substitution will be easier to track and attach to the genome. The power of generating and studying consomic animals is not only in measurable alteration of a quantitative trait, but in the ability to backcross the consomic rats to a parental strain and quickly give rise to congenic strains, narrowing the genomic interval that harbors the gene(s) of interest. Ultimately, the hope is to then study the tissue-specific expression, product(s), and mechanistic role the gene(s) of interest play in specific phenotypes.

In summary, the present study demonstrated phenotypic differences in ventilatory control among these four rat strains. Despite phenotypic differences in V˙e and f during hypoxia, the ventilatory response to hypoxia per se was not different among all strains. In addition, despite a severely blunted response to hypercapnia, BN rats tended to hyperventilate to a greater degree in response to submaximal exercise. Strain and sex differences in specific characteristics of ABs are also noted, allowing for the possibility of differences in the genetic determinants of these phenotypes. As a result, these data provide evidence that the genetic determinants of ventilatory control are specific to hypercapnia but also allow for the possibility of utilizing consomic rat strains to provide a more clear understanding of the genetic basis for ventilatory control mechanisms.

This research was supported by National Heart, Lung, and Blood Institute Grants HL-25739 and U01 HL-66579 and by the Veterans Affairs.

FOOTNOTES

- Address for reprint requests and other correspondence: H. V. Forster, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (E-mail: bforster@mcv.edu).
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- May 17, 2002;10.1152/japplphysiol.00019.2002

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