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Ventilatory Behavior and Carotid Body Morphology of Brown Norway and Sprague Dawley Rats

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Abstract

Differences in acute ventilatory behavior are associated with carotid body (CB) structural and immunohistologic profiles in some, but not all, reports. Brown Norway (BN) rats exhibit lower acute ventilatory responses to hypoxia and hypercapnia compared to Sprague Dawley (SD) rats. We hypothesized that BN rats possess CB with fewer glomus cells.

Ventilation was recorded in six-month-old BN and SD rats exposed to hypoxia-reoxygenation and hypercapnia. Extracted CBs were examined using H&E staining, and immunohistochemistry with antibodies specific for Tyrosine Hydroxylase (TH), Neural Nitric Oxide Synthase (nNOS), and Pyruvate Dehydrogenase (PD). Sections were analyzed for cell and immunostaining density.

SD displayed greater hypoxic and hypercapnic responses, and post-hypoxic short term potentiation, whereas BN exhibited post-hypoxic frequency decline. Contrary to our hypothesis, BN demonstrated a denser arrangement of glomus cells with a larger TH stained area (31.7% BN, 22.6% SD; $p < 0.0001$), and nNOS stained area (37.3% BN, 32.1% SD; $p = 0.01$).

Hence, respiratory phenotype does not correlate intuitively with these anatomic features.

1.0 Introduction

Ventilatory responses to hypercapnia and hypoxia are influenced by genetic diversity, independent of body mass, gender, or animal length (Hodges et al., 2002; Strohl et al., 1997). For instance, in comparison to the Sprague Dawley (SD) rat, the Brown Norway (BN) rat exhibits less pronounced increases in minute ventilation (V_E), tidal volume (V_T),

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and frequency (f) when exposed to hypoxia and hypercapnia (Hodges et al., 2002; Subramanian et al., 2007). Hence, studies limited to one strain of a species may not represent the total “physiology” of that species, nor be immediately applicable to a genetically diverse human population.

The carotid body (CB), in addition to its role as the chief peripheral chemoreceptor for acute changes in oxygen tension (Lopez-Barneo et al., 2008), provides information to the pons and medulla concerning peripheral pH and pCO₂ (Garcia-Fernandez et al., 2007; Peers and Buckler, 1995). The CB parenchyma consists of neuron-like chemosensory cells known as Type I, or Glomus cells, and glia-like sustentacular cells known as Type II cells. Numerous molecular mechanisms and messengers are implicated in the response of the glomus cell to hypoxia and are implicated in intra-species differences (Lopez-Barneo et al., 2008).

Nitric oxide (NO) is a gaseous molecule inhibiting carotid body responsiveness at low concentrations (Mosqueira and Iturriaga, 2002). Antagonism of nitric oxide synthase (NOS) with the competitive inhibitor N^G-nitro-L-arginine methyl Ester (L-NAME) increases the response of the carotid body to hypoxia and hypercapnia (Gozal et al., 1996; Valdes et al., 2003). Furthermore, mice with specific knockout mutations of neural nitric oxide synthase (nNOS) exhibit an augmented hypoxic response (Kline and Prabhakar, 2000). Our laboratory demonstrated that L-NAME administration results in greater increases in resting minute ventilation and hypercapnic response in the SD compared to the BN. Furthermore, the Dejours phenomenon (post-hypoxic decline in minute ventilation) and post-hypoxic frequency decline (PHFD) were observed in SD animals following L-NAME administration. Both Dejours phenomenon and PHFD were absent in the SD with vehicle administration (Subramanian et al., 2002). There are several studies examining nNOS protein expression in the carotid body but none describing strain differences (Dvorakova and Kummer, 2005; Leite et al., 2010; Li et al., 2010).

Other morphologic characteristics of the CB have been associated with hypoxic responsiveness. The density of glomus cells is inferred as a mechanism for differences. A mouse strain with lower hypoxic responsiveness (A/J) was found to possess a carotid body which was smaller with a lower percentage of CB surface area staining positive for tyrosine hydroxylase (TH) than a mouse strain with a more pronounced hypoxic response (Yamaguchi et al., 2003). TH catalyzes the rate limiting step of catecholamine synthesis. As type I or Glomus cells are the only catecholaminergic cells within the carotid body, staining for TH probes for differences in glomus cell density (Oomori et al., 1994). Mitochondrial inhibitors reduce carotid body oxygen sensation, leading to speculation that their relative abundance may underlie disparate hypoxic responses (Lopez-Barneo et al., 2008). The alpha-1 subunit of pyruvate dehydrogenase (PD) is a nuclear encoded protein found in the mitochondrial matrix, and it is a marker for mitochondrial density (Stacpoole et al., 2003; Strumilo, 2005).

Here it is postulated that morphologic and immunohistologic disparities in the carotid bodies of the two strains may be predicted from their functional differences in ventilatory responsiveness to hypoxia and hypercapnia.

2.0 Methods

2.1 Animals

Animals were purchased from Harlan Sprague–Dawley, Indianapolis, IN, and housed in the Animal Facility of the Louis Stokes DVA Medical Center. Adult SD (SD/Harlan) rats ($n = 18$ males) and Brown Norway (BN/Harlan) rats ($n = 14$ males) were housed in the same room under 12:12:L:D cycles and fed the same rat chow and water ad libitum at least two weeks prior to testing. As described in 2.2 and 2.3, sixteen animals ($n=10$, SD and $n=6$, BN) were used for plethysmography testing, and sixteen animals ($n=8$ for both strains) were used for CB morphology analysis. Use of animals in this study was performed as outlined in the *Guide to the Care and Use of Laboratory Animals* written by the Institute of Laboratory Animal Resources, National Research Council. This study and its procedures were also approved by the Louis Stokes Veterans Affairs Medical Center's Institutional Animal Care and Use Committee.

2.2 Plethysmography Testing

As hitherto the phenotypic differences between BN and SD had been investigated in animals aged 12–16 weeks (Subramanian et al., 2002), 6 month (~26 week) old animals were subjected to plethysmography testing prior to carotid body extraction from age-matched peers in the current study. Ventilation was recorded via whole body plethysmography (BN $n = 6$, SD $n = 10$) using an apparatus which our lab has used previously (Subramanian et al., 2007). Testing occurred between 9am and 12pm to avoid circadian effects, and studies were performed at ambient temperatures. Each rat was placed in the plethysmography apparatus for two hours the day before testing and was acclimated for one hour in the chamber on the day of testing. Following acclimatization, one minute of resting breathing was recorded. Animals then experienced five minutes of hypoxia which was either poikilocapnic (8% O₂, bal-N₂) or isocapnic (10% O₂, 3% CO₂, bal-N₂) followed by a five minute reoxygenation period with 100% O₂. Following this, a twenty minute room-air rest period occurred. Thereafter, animals were exposed to hypercapnia (7% CO₂, 93% O₂) for five minutes, then another twenty minute rest period. Finally, the animal experienced another five minute hypoxic exposure (with the mixture to which it had not previously been exposed) followed by five minutes of reoxygenation with 100% O₂. The order of poikilocapnic and isocapnic challenges were alternated such that half of each strain was exposed to the poikilocapnic challenge first, and vice versa.

2.3 Analysis of Respiratory Data

Plethysmography data was scored using a program custom written for our lab (Breath Detect) which was created to be compatible with data obtained in Lab View 7.1 (National Instruments, Austin, TX). Data were scored from ten second continuous recordings during the first minute of each measurement period. Sniffs and sighs were excluded from analysis.

The values of f , V_T , and V_E (with V_T and V_E normalized for weight) were compared between strains using a student's t-test. To evaluate for short term potentiation (STP) and post-hypoxic frequency decline (PHFD), baseline f was compared to f during the first minute of reoxygenation using a paired samples t-test. For each of these comparisons,

significance was attained at $p = 0.05$. Values are expressed as mean $\pm$ standard error of the mean (SEM).

2.4 Tissue Collection

CB tissue was collected from age matched (6 month old) animals which were not tested in the plethysmography chamber ($n = 8$ per strain); this enabled avoidance of the confounding effects of hypoxia on carotid body tyrosine hydroxylase expression (Kato et al., 2010). For both the BN and SD strains, six-month old male rats ($n=8$) were used. Following sacrifice via CO_2 asphyxiation, an initial midline neck incision was made, the investing fascia of the neck was dissected, and the carotid artery was located $\sim 1\text{mm}$ lateral to the trachea. The carotid artery and carotid bifurcation were mobilized from the overlying fascia. The internal carotid and external carotid arteries were ligated 1–2 mm superior to the bifurcation, and the common carotid artery was ligated 5 mm inferior to the bifurcation. The samples were collected within five minutes of death and were placed in 10% Buffered Formalin Phosphate.

2.5 Morphological Analysis

One carotid body per animal ($n=8$ per strain) was used for analysis of morphology. Following 24-hour formalin fixation, CBs were stored in 0.01 M phosphate buffered saline at 4°C . Samples were embedded in paraffin and serial $5\text{ }\mu\text{m}$ sections were obtained. H&E staining was performed on $5\text{ }\mu\text{m}$ sections at five separate levels. Each $5\text{ }\mu\text{m}$ section was separated from the next by $40 - 50\text{ }\mu\text{m}$.

Images were obtained using a brightfield microscope and high resolution digital camera (Olympus BX51, Olympus America Inc., Center Valley, PA). Individual photomicrographs were compiled and cropped in ImageJ (ImageJ 1.43t) which is a well-regarded Java-based freeware developed at the NIH and used for image processing by investigators in the life sciences (Rasband, 1997 – 2010). Compiled images of carotid body sections were cropped in ImageJ prior to analysis such that only the carotid body was obtained. Peripheral vasculature, adipose tissue, connective tissue and other stromal elements were excluded. A random selection of ten images cropped by the primary operator were blindly retested by a second operator, and the samples' surface area differed by less than 4%. Estimates of cell density in these samples were obtained using the cell counting feature of ImageJ, and the number of cells were compared to total cross-sectional area of carotid body to obtain an estimation of cell density. Identical thresholds for circularity, color, and cell size were used for all sections imaged. Values are expressed as mean $\pm$ standard error of the mean (SEM).

2.6 Immunohistochemistry

Immunohistochemistry using antibodies against nNOS, TH, and PD proteins was also employed. The three largest cross sections of carotid body for each animal were used for immunohistochemistry. For nNOS, TH, and PD staining, the slides were baked at 60°C , deparaffinized in xylene, and rehydrated in graded ethanol baths. Antigen retrieval was performed by incubating the samples in steam at 98°C in *Rodent Decloaker Buffer* (Biocare Medical, Concord, CA). Endogenous peroxidase was blocked with *Peroxidized 1* and *Rodent Block* (BioCare Medical). For staining of nNOS, a rabbit derived anti-nNOS

polyclonal antibody (1:250) (BD Biosciences, San Diego, CA) was used; for TH staining, the samples were incubated overnight with rabbit made polyclonal anti-TH antibody (1:1000)(Chemicon/Millipore, Billerica, MA). A rabbit derived anti-PD alpha1 antibody, 1:200, (Cell signaling technology, Danvers, MA) was used. This alpha 1 subunit is nuclear encoded and found in the mitochondrial matrix (Stacpoole et al., 2003; Strumilo, 2005). Anti-Rabbit IgG was used as a negative control, and samples were analyzed following staining to confirm lack of staining (slides available upon request). Following incubation, primary antibodies were marked with MACH4 HRP-Polymer (Biocare Medical) chromogen. Samples were counterstained with hematoxylin.

Images stained for immunohistochemistry were analyzed for cell density using the ImageJ program as described above. Due to the selective staining of Glomus cells using tyrosine hydroxylase, a more confident cell density of these cells was obtained than would be possible with H&E staining. For nNOS, TH, and PD samples, a stained to unstained ratios of each section of carotid body were used to calculate the ratio of TH, nNOS, and PD stained area to total CB area. Strains were compared via student's t-test. Values are expressed as mean $\pm$ standard error of the mean (SEM).

3.0 Results

3.1 Animal mass

The average body mass of the BN animals tested for plethysmography data was 343 ± 7 g (mean $\pm$ SEM), and that of the SD animals was 514 ± 18 g ($p < 0.0001$). For histologic studies, at the time of sacrifice, BN rats were 363 ± 9 g, and SD donor rats were 495 ± 15 g ($p < 0.0001$).

3.2 Results of plethysmography challenges

At baseline, the SD and BN had similar f , the BN animals possessed a slightly larger V_T although there was no difference in V_E (Figure 1).

During both isocapnic and poikilocapnic hypoxia and reoxygenation, the SD demonstrated a significantly higher respiratory frequency than the BN. Similar to baseline respiration, BN demonstrated a larger tidal volume when normalized for mass during reoxygenation following both poikilocapnic and isocapnic hypoxia. The SD demonstrated a significantly higher V_E during isocapnic reoxygenation, and a trend towards a larger V_E during isocapnic hypoxia ($p = 0.065$). SD also demonstrated a significantly larger V_E during hypercapnia (Figure 1).

In order to assess for STP and PHFD, frequency recorded during the first minute of reoxygenation following hypoxia was compared to baseline respiratory frequency. During the first minute of reoxygenation following isocapnic hypoxia, the BN displayed PHFD, a lower f than at baseline, (baseline 109.1 ± 10.9 vs. 74.1 ± 3.5 breaths/min, $p=0.022$), and the SD displayed STP, a greater f than at baseline (baseline 109.7 ± 6.8 vs. 169.5 ± 18.0 breaths/min, $p = 0.006$). Following poikilocapnic hypoxia, SD demonstrated STP (baseline 109.7 ± 6.8 vs. 208.4 ± 33.0 breaths/min, $p=0.008$), but no change from baseline was seen in the BN (baseline 109.1 ± 10.9 vs 112.0 ± 29.7 breaths/min, $p=0.902$).

3.3 Carotid Body Morphology

Upon gross inspection of H&E stained CB sections, no obvious differences in shape or size were immediately observed. The carotid body was found to be an ellipsoid structure with numerous glomus cells ~ 10 μ m in diameter, basophilic in color, surrounded by sustentacular cells which possessed thin darkly stained nuclei (Figures 2,3). In both species, a close spatial association between the CB, the internal carotid artery and the carotid sinus nerve was observed. In numerous sections of carotid sinus nerve, glomus cells were observed, and nervous tissue was frequently found enveloped by carotid body parenchyma (eg. Figure 2a). Groupings of glomus cells were observed which varied in size and shape (Figures 2, 3).

No significant differences between strains were found in the area of carotid body cross-sections, cell density, or cell area (Table 1).

In analyses performed on sections stained for TH (Figure 4), BN demonstrated larger and more concentrated glomus cells than SD (Table 2, Figure 4).

With nNOS staining, the glomus cells and nerves in both strains were selectively stained compared to the surrounding tissue (Figure 4). The BN exhibits a larger fraction of carotid body surface area staining positively for nNOS; but, because of the larger glomus cell surface area in the BN, no difference was found in the density of cells stained with nNOS (Table 2).

PD stained samples were obtained to evaluate mitochondrial density (Figure 4). No significant differences between the two strains were found for the percentage of carotid body surface area stained with PD or the density of PD stained cells (Table 2).

4.0 Discussion

4.1 Respiratory Physiology among Strains

Consistent with previous studies (Hodges et al., 2002; Subramanian et al., 2007), genetic background (strain) affected ventilatory functional responses in rats. In particular, ventilatory differences between strains were marked in the first minute of gas challenges—periods where acute sensation by the CB might seem to be most important. Also consistent with previous reports (Hodges et al., 2002; Subramanian et al., 2007), this study found a significantly diminished hypercapnic response in the BN compared to the SD.

4.2 Carotid body morphology

Despite demonstrating decreased hypoxic and hypercapnic ventilatory responses, the BN unexpectedly possessed larger and more densely arranged glomus cells than the SD. In a prior study, the ratio of TH+ area to total cross sectional area correlated positively with greater hypoxic responsiveness in the A/J and DBA/2J mouse strains (Yamaguchi et al., 2003). In contrast, herein the ratio of TH+ area to total area was not shown to correlate with hypoxic responsiveness in the two rat strains studied. Furthermore, recent work from our laboratory has demonstrated similar results in mice; the A/J mouse was found to exhibit both a decreased CB glomus cell density and an increased hypoxic ventilatory response in comparison to the C57/BL6 (Chai et al., 2011).

CB volume, while unlikely, might still underlie the differences between the BN and SD. CB volume was reported to vary in the A/J and DBA/2J mouse comparison performed by Yamaguchi et al with the DBA/2J possessing a much larger carotid body. However, significant differences in CB volume track with hypoxic responsiveness in some comparisons but not others (Pawar et al., 2008; Wenninger et al., 2006). However, in the present study the en bloc extraction precluded weighing an isolated carotid body, as the extraneous tissue made volumetric analysis problematic.

A larger percentage of total CB area stained positively for nNOS in the BN. This observed difference could represent an opportunity for increased *in vivo* NO synthesis in the CB of the BN and contribute to the differential response of these strains to administration of the NOS inhibitor L-NAME. Previously, L-NAME resulted in a larger augmentation of SD baseline respiratory frequency than that seen in the BN (Subramanian et al., 2002). Decreased nNOS levels in the SD may cause that strain to possess greater sensitivity to the inhibitory effects of L-NAME. Also, acute L-NAME administration enhances hypoxic and hypercapnic ventilatory response in SD rats (Gozal et al., 1996), and mice possessing nNOS deletions demonstrate augmented responses to acute hypoxia (Kline and Prabhakar, 2000). Therefore, a relatively diminished capacity for NO synthesis in the SD may also explain its relatively greater hypoxic and hypercapnic responses. However, to our knowledge, the anatomic location or network systems for these effects is not known, and it is possible that these nNOS-related ventilatory effects are due to differential activity in the pontine or medullary respiratory centers, with or without additional carotid body influence.

Due to similarities in PD staining, it is unlikely that mitochondrial density plays a role in the variation of hypoxic responsiveness between these strains.

4.3 Limitations and Future Directions

In this study, a greater acute hypoxic response seen in the SD is used as evidence of greater CB chemosensitivity. However, it is possible that the BN do indeed possess greater than or similar CB afferent activity in relation to the SD, but such afferent activity is simply processed differently in the medulla. Nerve recording from the isolated and severed carotid sinus nerve can be used to measure afferent activity of the CB in response to hypoxia (Li et al., 2010). Such a technique could be used to compare CB activity of the SD and BN, isolating CB functions from those of central processing for respiratory control. Should these two strains lack significant differences in CB afferent activity, one could implicate the targets of CB afferent signaling as a critical site for integration (Spyer and Gourine, 2009) and strain variation.

Furthermore, we assume in this study that the smaller percentage of CB surface area staining for nNOS in the SD is correlated with diminished capacity for NO production. Further investigation using gene and protein expression of nNOS as well as measurements of *in vivo* NO production will be needed to explain the disparity in their response to L-NAME (Subramanian et al., 2002).

4.4 Conclusions

The current study illustrates that differences in CB structure are strongly determined by genetic background. Contrary to inferences of physiologic or morphologic studies limited to one or to a limited number of strains, these differences in CB structure do not intuitively correlate with disparities in the ventilatory phenotype. Indeed, inefficiency of chemosensitivity in the more prevalent BN glomus cells, or differences in integration of carotid body afferent activity may underlie the apparent differences in hypoxic and hypercapnic sensitivity. This complexity will confound human studies that do not acknowledge genetic diversity as a significant causal feature for trait variation.

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References

- Chai S, Gillombardo CB, Donovan L, Strohl KP. Morphological differences of the carotid body among C57/BL6 (B6), A/J, and CSS B6A1 mouse strains. *Respir Physiol Neurobiol*. 2011
- Dvorakova MC, Kummer W. Immunohistochemical evidence for species-specific coexistence of catecholamines, serotonin, acetylcholine and nitric oxide in glomus cells of rat and guinea pig aortic bodies. *Ann Anat*. 2005; 187:323–331. [PubMed: 16163845]
- Garcia-Fernandez M, Ortega-Saenz P, Castellano A, Lopez-Barneo J. Mechanisms of low-glucose sensitivity in carotid body glomus cells. *Diabetes*. 2007; 56:2893–2900. [PubMed: 17827405]
- Gourine AV. On the peripheral and central chemoreception and control of breathing: an emerging role of ATP. *J Physiol*. 2005; 568:715–724. [PubMed: 16141266]
- Gozal D, Torres JE, Gozal YM, Littwin SM. Effect of nitric oxide synthase inhibition on cardiorespiratory responses in the conscious rat. *J Appl Physiol*. 1996; 81:2068–2077. [PubMed: 8941531]
- Hodges MR, Forster HV, Papanek PE, Dwinell MR, Hogan GE. Ventilatory phenotypes among four strains of adult rats. *J Appl Physiol*. 2002; 93:974–983. [PubMed: 12183493]
- Kato K, Yamaguchi-Yamada M, Yamamoto Y. Short-term hypoxia increases tyrosine hydroxylase immunoreactivity in rat carotid body. *J Histochem Cytochem*. 2010; 58:839–846. [PubMed: 20530461]
- Kline DD, Prabhakar NR. Peripheral chemosensitivity in mutant mice deficient in nitric oxide synthase. *Adv Exp Med Biol*. 2000; 475:571–579. [PubMed: 10849697]
- Leite MS, Damaceno-Rodrigues NR, Simone MR, Santos AB, Bueno HM, Battlehner CN, Mauad T, Caldini EG, Saldiva PH. Structural alterations in adult rat carotid bodies exposed to hyperbaric oxygenation. *Undersea Hyperb Med*. 2010; 37:419–432. [PubMed: 21226393]
- Li YL, Zheng H, Ding Y, Schultz HD. Expression of neuronal nitric oxide synthase in rabbit carotid body glomus cells regulates large-conductance Ca^{2+} -activated potassium currents. *J Neurophysiol*. 2010; 103:3027–3033. [PubMed: 20357072]
- Lopez-Barneo J, Ortega-Saenz P, Pardal R, Pascual A, Piruat JJ. Carotid body oxygen sensing. *Eur Respir J*. 2008; 32:1386–1398. [PubMed: 18978138]
- Mosqueira M, Iturriaga R. Carotid body chemosensory excitation induced by nitric oxide: involvement of oxidative metabolism. *Respir Physiol Neurobiol*. 2002; 131:175–187. [PubMed: 12126919]
- Oomori Y, Nakaya K, Tanaka H, Iuchi H, Ishikawa K, Satoh Y, Ono K. Immunohistochemical and histochemical evidence for the presence of noradrenaline, serotonin and gamma-aminobutyric acid in chief cells of the mouse carotid body. *Cell Tissue Res*. 1994; 278:249–254. [PubMed: 8001081]

- Pawar A, Peng YJ, Jacono FJ, Prabhakar NR. Comparative analysis of neonatal and adult rat carotid body responses to chronic intermittent hypoxia. *J Appl Physiol.* 2008; 104:1287–1294. [PubMed: 18187605]
- Peers C, Buckler KJ. Transduction of chemostimuli by the type I carotid body cell. *J Membr Biol.* 1995; 144:1–9. [PubMed: 7595937]
- Rasband, WS. ImageJ. 1997–2010. <http://rsb.info.nih.gov/ij/>
- Spyer KM, Gourine AV. Chemosensory pathways in the brainstem controlling cardiorespiratory activity. *Philos Trans R Soc Lond B Biol Sci.* 2009; 364:2603–2610. [PubMed: 19651660]
- Stacpoole PW, Owen R, Flotte TR. The pyruvate dehydrogenase complex as a target for gene therapy. *Curr Gene Ther.* 2003; 3:239–245. [PubMed: 12762482]
- Strohl KP, Thomas AJ, St Jean P, Schlenker EH, Koletsky RJ, Schork NJ. Ventilation and metabolism among rat strains. *J Appl Physiol.* 1997; 82:317–323. [PubMed: 9029232]
- Strumilo S. Short-term regulation of the mammalian pyruvate dehydrogenase complex. *Acta Biochim Pol.* 2005; 52:759–764. [PubMed: 16025163]
- Subramanian S, Dostal J, Erokku B, Han F, Dick TE, Strohl KP. Domperidone and ventilatory behavior: Sprague-Dawley versus Brown Norway rats. *Respir Physiol Neurobiol.* 2007; 155:22–28. [PubMed: 16781202]
- Subramanian S, Erokku B, Han F, Dick TE, Strohl KP. L-NAME differentially alters ventilatory behavior in Sprague-Dawley and Brown Norway rats. *J Appl Physiol.* 2002; 93:984–989. [PubMed: 12183494]
- Valdes V, Mosqueira M, Rey S, Del Rio R, Iturriaga R. Inhibitory effects of NO on carotid body: contribution of neural and endothelial nitric oxide synthase isoforms. *Am J Physiol Lung Cell Mol Physiol.* 2003; 284:L57–68. [PubMed: 12388352]
- Wenninger JM, Olson EB, Wang Z, Keith IM, Mitchell GS, Bisgard GE. Carotid sinus nerve responses and ventilatory acclimatization to hypoxia in adult rats following 2 weeks of postnatal hyperoxia. *Respir Physiol Neurobiol.* 2006; 150:155–164. [PubMed: 15978876]
- Yamaguchi S, Balbir A, Schofield B, Coram J, Tankersley CG, Fitzgerald RS, O'Donnell CP, Shirahata M. Structural and functional differences of the carotid body between DBA/2J and A/J strains of mice. *J Appl Physiol.* 2003; 94:1536–1542. [PubMed: 12626474]

Figure 1.

Bar graphs of f (top), V_T (middle), and V_E (bottom) for each strain. B- Baseline, IH- Isocapnic Hypoxia, PH- Poikilocapnic Hypoxia, R-Reoxygenation, HC-Hypercapnia. Error bars represent $\pm$ standard error.

*-p < 0.05, **-p<0.01, ***-p<0.001.

Figure 2. Photomicrographs of representative BN carotid body stained with H&E at 10x and 20x magnification. Location of 20x image is outlined in black on the 10x image. Internal Carotid Artery (ICA) and Carotid Sinus Nerve (CSN) are labeled in 10x image. Scale bar (0.5 mm) is present in lower right lower corner of images.

Fig 3a SD 10x

Fig 3b SD 20x

Figure 3. Photomicrographs of representative SD carotid body stained with H&E at 10x and 20x magnification. Location of 20x image is outlined in black on the 10x image. Internal Carotid Artery (ICA) and Carotid Sinus Nerve (CSN) are labeled in 10x image. Scale bar (0.5 mm) is present in lower right lower corner of images.

Figure 4. Photomicrographs of BN (left column) and SD (right column) carotid bodies stained against TH (top row), nNOS (center row), and PD (bottom row) and counterstained with hematoxylin at 40x magnification. Scale bar (0.1 mm) is present in the right lower corner of all images.

Table 1

Cytological Metrics from SD and BN H&E.

	Strain		p-value
	BN	SD	
Avg Cell Area ($1 \times 10^2 \mu\text{m}^2$)	1.75 ± 0.16	1.52 ± 0.16	0.3210
Cells Density ($1 \times 10^3 \text{ cells/mm}^2$)	2.18 ± 0.05	2.33 ± 0.10	0.2170
Cell Area/Total Area %	35.0 ± 2.0	32.8 ± 2.2	0.4789
Average CB Area/Slice mm^2	1.451 ± 0.177	1.592 ± 0.240	0.6426

Figure 1. Values are expressed as mean $\pm$ standard error. No values were found to be significant at the p 0.05 level.

Table 2

Cytological Metrics from SD and BN TH, nNOS, and PD Staining.

	Strain		p-value
	BN	SD	
TH+ Area/Total Area %	31.7 ± 2.8^I	22.6 ± 3.5^I	<0.0001
TH+ Cells/Total Area (cells/mm2)	663 ± 52^I	606 ± 43^I	0.0302
nNOS + Area/Total Area %	37.3 ± 3.8^I	32.1 ± 3.1^I	0.0099
nNOS+ Cells/Total Area (cells/mm2)	583 ± 64	622 ± 63	0.2262
PD+ Area/Total Area %	11.9 ± 3.6	10.4 ± 2.2	0.3379
PD+ Cells/Total Area (cells/mm2)	386 ± 49	347 ± 31	0.5186

Table 2. Values are expressed as mean ± standard error.

^IValues significant at the p 0.05 level (also in bold).