Supporting Information

Peng et al. 10.1073/pnas.1322172111

Materials and Methods

Immunocytochemistry. Carotid bodies were harvested from anesthetized rats and perfused with heparinized saline followed by 4% (vol/vol) paraformaldehyde for 30 min. Carotid body sections (8 μm thick) were stained with monoclonal mouse anti-tyrosine hydroxylase antibody (1:2,000, Sigma), polyclonal rabbit anti-cystathionine-γ-lyase (CSE) antibody (S.H.S.; 1:200 dilution), or polyclonal rabbit Heme oxygenase 2 (HO-2) antibody (S.H.S.; 1:1,000 dilution), as previously described (1). Morphometric analysis of carotid bodies was performed as outlined earlier (2).

Immunoblot Assays. Immunoblot assays of HO-1 and HO-2 in liver microsomal fractions were performed as previously described (3) with polyclonal anti-HO-1 antibody (1:1,000; Enzo Life Sciences), polyclonal anti-HO-2 antibody (1:2,000; Abcam), or polyclonal anti-α-tubulin antibody (1:2,000; Sigma).

Drugs. The following drugs were used: CrM459 [CrMP, Cr(III) mesoporphyrin IX chloride; Frontier Scientific], CORM-2 ($[Ru(CO)_3\ Cl_2]$, tricarbonyldichlororuthenium (II) dimer; Sigma-Aldrich), and L-propargylglycine (L-PAG; Chem-Impex International).

Drug Treatments. In experiments assessing the effect of HO and CSE inhibitors, rats were either injected with Cr(III) meso porphyrin, an HO inhibitor (CrMP; 1 mg/kg in 0.2% DMSO, i.p.), alone or in combination with L-propargylglycine, a CSE inhibitor (L-PAG; 30 mg/kg in saline, i.p.). One hour after injection, rats were anesthetized and carotid bodies were harvested to measure sinus nerve activity. In the experiments assessing the effects of CO donor, ex vivo carotid bodies were superfused with CORM-2 (20 μM). In the experiments assessing the effect of HO inhibition on ventilatory adaptations, rats were administered CrMP (1 mg/kg; dissolved in 0.2% DMSO + peanut oil; volume, 200 μL; s.c.) 1 h before exposure to HH. In the experiments assessing the effects of HO or CSE inhibition or CO donor on glomus cell secretory responses, cells were treated with CrMP (10 μM), L-PAG (10 μM), or CORM-2 (20 μM), respectively, 30 min before amperometric measurements.

Data Analysis. Average data are presented as mean ± SEM. Statistical significance was assessed by either ANOVA or two-way ANOVA with repeated measures followed by Tukey's test. P values < 0.05 were considered significant.

1. Peng YJ, et al. (2010) H2S mediates O2 sensing in the carotid body. *Proc Natl Acad Sci USA* 107(23):10719–10724.
2. Kline DD, Peng YJ, Manalo DJ, Semenza GL, Prabhakar NR (2002) Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 alpha. *Proc Natl Acad Sci USA* 99(2):821–826.
3. Yuan G, et al. (2013) Mutual antagonism between hypoxia-inducible factors 1α and 2α regulates oxygen sensing and cardio-respiratory homeostasis. *Proc Natl Acad Sci USA* 110(19):E1788–E1796.

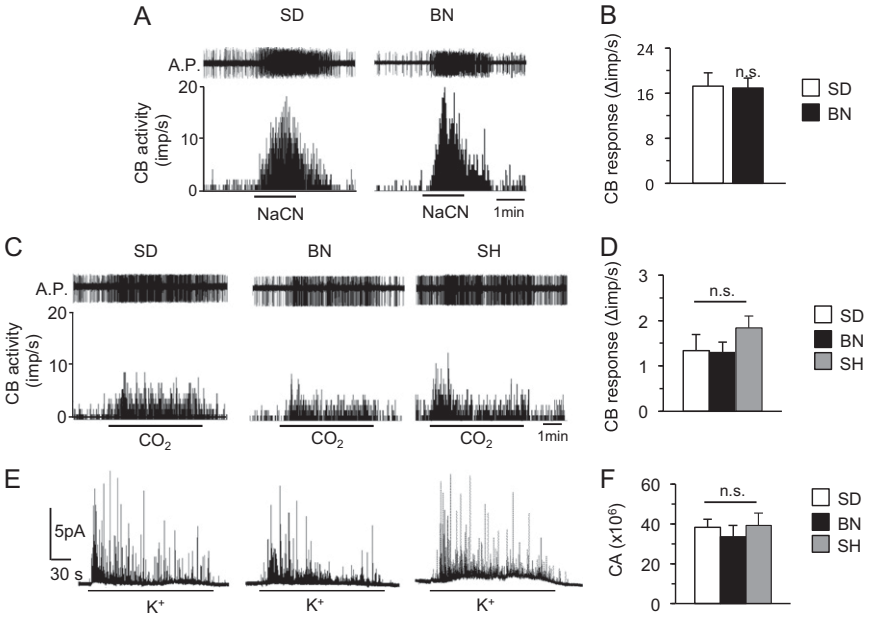

Fig. S1. Comparable response to NaCN, CO_2, and high K^+ among three rat stains. (*A* and *B*) Examples of carotid body sensory response to NaCN (at black bar; 3 μg/mL) in Sprague-Dawley (SD) rats and Brown-Norway (BN) rats (*A*). Average (mean ± SEM) data measured as the difference in response between baseline and NaCN (Δimp/s; *B*). (*C* and *D*) Examples of carotid body sensory response to CO_2 (P_{CO2} = 58–60 mm Hg) in SD, BN, and Spontaneous Hypertensive (SH) rats (*C*). Average data (mean ± SEM) measured as the difference in response between baseline and CO_2 (Δimp/s; *D*). In *A* and *C*, action potentials (A.P.) from the sinus nerve and integrated carotid body sensory activity (CB activity) are presented as impulses per second (imp/s). (*E* and *F*) Examples of glomus cell catecholamine (CA) secretory responses to 40 mM KCl in SD, BN, and SH rats (*E*) and average data (mean ± SEM) of CA secretion. n = 6–7 rats in each group and 11–13 cells in each group. n.s. $P > 0.05$ (i.e., not significant).

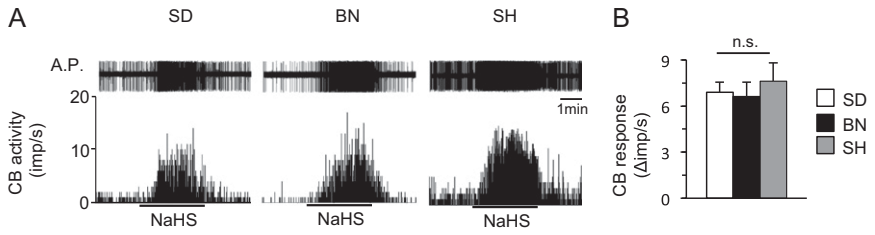

Fig. S2. Comparable sensory excitation to NaHS among three rat stains. (*A*) Examples of carotid body responses to NaHS (at black bar; 50 μM), an H_2S donor, in SD, BN, and SH rats. Action potentials (A.P.) from the sinus nerve and integrated carotid body sensory activity (CB activity) are presented as impulses per second (imp/s). (*B*) Average (mean ± SEM) data of carotid body sensory response to NaHS presented as difference in response between baseline and NaHS (Δimp/s). n = 5–6 rats of each strain. n.s. $P > 0.05$ (i.e., not significant).

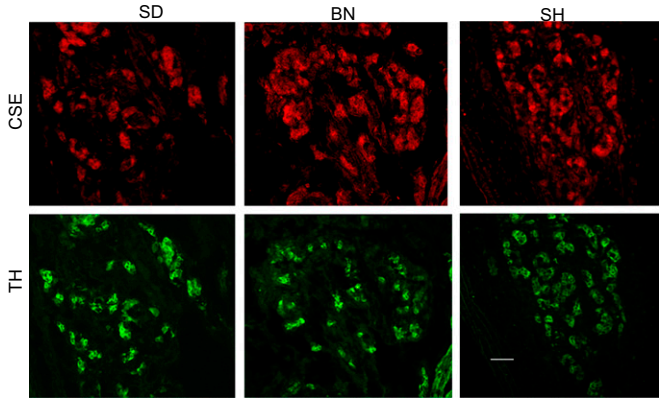

Fig. S3. Comparable immunocytochemical expression of CSE among three rat stains. Examples of CSE expressions in SD, BN, and SH carotid bodies. Carotid body sections were stained with antibodies specific for CSE and tyrosine hydroxylase, a marker of glomus cells. Horizontal bar represents 20 μm.

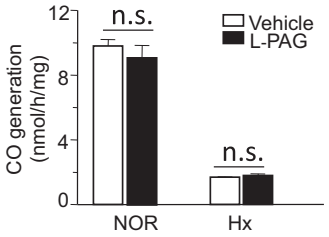

Fig. S4. L-PAG reduces CO generation in SD rats. Effect of L-PAG (10 μM), a CSE inhibitor, on CO generation in SD rat carotid body during normoxia (Nx) and hypoxia (Hx). Hx = P_{O2} ~30 mm Hg. Data are mean ± SEM from n = 5 experiments. n.s. $P > 0.05$ (i.e., not significant).

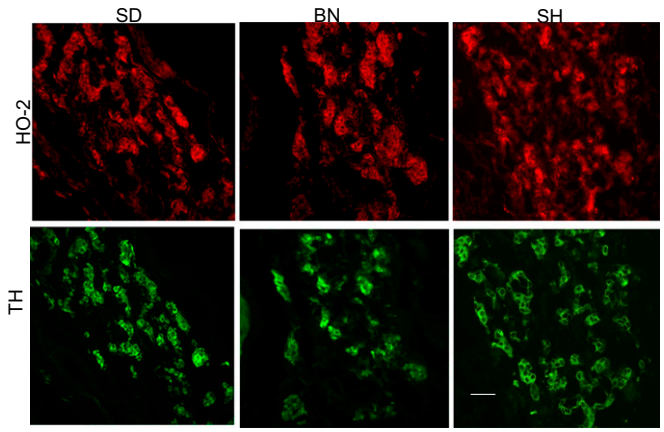

Fig. S5. Comparable immunocytochemical expression of HO-2 among three rat stains. Examples of hemeoxygenase 2 (HO-2) expressions in SD, BN, and SH carotid bodies. Carotid body sections were stained with antibodies specific for HO-2 and tyrosine hydroxylase, a marker of glomus cells. Horizontal bar represents 20 μm.

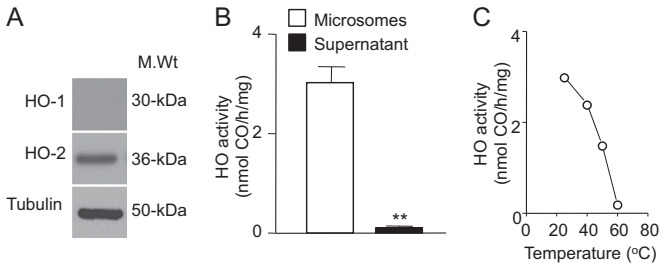

Fig. S6. HO-2 is the predominate CO generating enzyme. (*A*) Western blot analysis of the enzymes HO-1 and HO-2 in liver microsomal fractions from SD rats. Tubulin protein was analyzed as loading control. (*B*) Average (mean ± SEM) data of CO generation measured in liver microsomal and supernatant fractions from SD rats. $n = 5$. (*C*) Average (mean ± SEM) data of effects of temperature on CO generation in liver microsomes in SD rats. $n = 3$. $^{**}P < 0.01$.

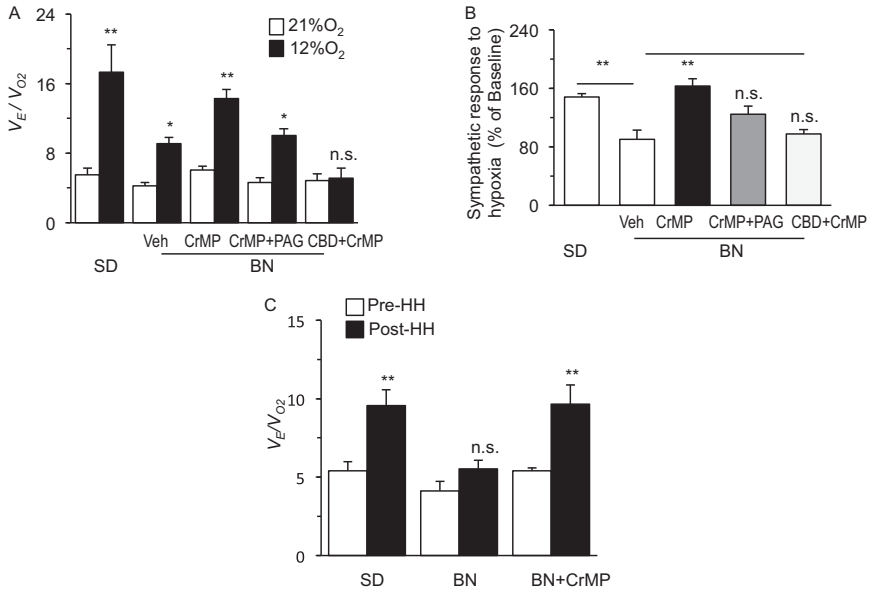

Fig. S7. HO inhibition restores chemosensory reflex and ventilatory adaptation in BN rats. (*A–C*) Average (mean ± SEM) data of (*A*) minute ventilation (V_E) of 21% and 12% inspired O_2 as ratio of O_2 consumption (V_{O2}), (*B*) splanchnic sympathetic nerve responses to 12% inspired O_2 (Hx; black bar in Fig. 5*B*), and (*C*) V_E as ratio V_{O2} before (pre) and after (post) exposure to hypobaric hypoxia (HH; 0.35 atmospheres) in SD and BN rats treated with either vehicle or the HO inhibitor CrMP. $n = 7–8$ rats in each group. $^{*}P < 0.05$; $^{**}P < 0.01$; n.s. $P > 0.05$ (i.e., not significant).

Table S1. Body weight and blood pressure in three rat stains

	SD	BN	SH
BW (g)	126 ± 3	108 ± 2*	105 ± 2*
SBP (mm Hg)	110 ± 3	108 ± 4	112 ± 3
DBP (mm Hg)	74 ± 2	73 ± 3	76 ± 2
MBP (mm Hg)	86 ± 2	85 ± 3	88 ± 2

Average data of body weights, SBP, DBP, and MBP in 5–6-wk-old SD, BN, and SH rats. Data are mean ± SEM. $n = 7$–8 rats in each group.
*$P < 0.05$.

Table S2. O_2 consumption and CO_2 production in BN rats

	BN		BN + CrMP	
Metabolic variables	21% O_2	12% O_2	21% O_2	12% O_2
VO_2 (ml/g·min)	0.043 ± 0.003	0.030 ± 0.002	0.048 ± 0.004	0.030 ± 0.003
VCO_2 (ml/g·min)	0.018 ± 0.001	0.016 ± 0.002	0.019 ± 0.001	0.017 ± 0.002

Average data of O_2 consumption (V_{O2}) and CO_2 production (V_{CO2}) in BN rats treated with either vehicle or a heme oxygenase inhibitor (CrMP; 1 mg/kg, i.p.) during inspiration of 21% and 12% O_2.