Supporting Information

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SI Results

Results of Brainstem *en Bloc* Preparation Experiments. Duration of the inspiratory burst (TI) increased similarly in all groups of mice when preparations were superfused with anoxic artificial cerebrospinal fluid (aCSF) as compared to the control condition. Thus, *Task2*$^{-/-}$ mice display some responsiveness to hypoxia as evidenced by the increase in burst duration even though the frequency of bursts is not affected by hypoxia. In all genotypes, TI was not affected by acidosis and alkalosis. Amplitude of C4 bursts was found to increase significantly in all groups of mice only in response to acidosis. No significant changes could be detected in surface area of C4 bursts in response to any given test solution (Table S1).

Effect of Hypoxia on Task2 mRNA Expression. Recently, evidence has been provided for a negative regulation of the human *TASK2* promotor by hypoxia in cell culture experiments (1). Such a decrease in murine *Task2* transcription could be an attractive explanation for the adaptation to chronic hypoxia observed in wild-type mice. To test this hypothesis, we measured Task2 mRNA in control animals and adapted animals after 24 h hypoxia (10% O_2). Surprisingly, Task2 mRNA was not down-regulated by hypoxia in mouse kidney and brainstem. Similarly, Task1 (a Task2-related K^+ channel expressed in the brainstem) was not regulated by hypoxia at a transcriptional level. In the same mRNA samples, erythropoietin showed the expected changes in response to hypoxic conditions (Fig. S1). Therefore, down-regulation of *Task2* gene transcription appears not to underlie the adaptation upon chronic hypoxia in wild-type mice.

RNA was isolated from mouse kidney and brainstem using the RNeasy mini kit (Qiagen). For reverse transcription, reverse transcriptase (Promega) was used according to the manufacturer's protocol. Real-Time PCR was performed with a LightCycler System 2.0 (Roche) using the SYBR Green PCR kit (Qiagen). Primers and conditions for Real-Time PCR were the following: mouse erythropoietin (NM_007942)—forward primer AGAATGGAGGTGGAAGAACAG and reverse primer TGTCTATATGAAGCTGAAGGGT (annealing temperature 57 °C); mouse task2 (NM_021542)—forward primer GCTTTGGGGACTTTGTGG and reverse primer AAAGAGGGACAGCCAAGC (annealing temperature 55 °C); and mouse β-actin—forward primer CCACCGATCCACACAGAGTACTT and reverse primer GACAGGATGCAGAAGGAGATTACTG (annealing temperature 55 °C). To test for transcriptional regulation of task2 by hypoxia, wild-type mice were kept on 10% O_2 for 24 h.

Effects of Metabolic Acidosis. Hypoxia is known to provoke respiratory alkalosis that could contribute to hypoxic depression in wild-type mice. Such an alkalosis could be diminished in mutant mice due to the renal metabolic acidosis (2). Therefore, long-term hypoxia was repeated in acidotic animals in which blood pH was reduced by 0.2 pH units by adding 50 mM NH_4Cl to the drinking water 12 h before the experiments. When exposed to hypoxia, the ventilatory depression was still observed in acidotic wild-type animals and was absent in *Task2*$^{-/-}$ mice (Fig. S2A). Therefore, slight metabolic acidosis is not sufficient to explain the lack of hypoxic ventilatory depression that we observed in *Task2*$^{-/-}$ mice. The CO_2 response of wild-type animals was slightly changed by metabolic acidosis but did not show the same disturbance of CO_2 sensitivity with strong responses at very low CO_2 as observed in knockout animals (Fig. S2B).

Reactive Oxygen Species Activate hTASK2 Currents. The lack of central hypoxic response in plethysmographic and *en bloc* experiments suggested that, in addition to their pH sensitivity, Task2 currents are also sensitive to partial pressure of O_2. Hypoxia is known to induce reactive oxygen species (ROS) generation (3, 4). Heterologous expression studies in *Xenopus* oocytes have shown that Task2 is activated by ROS generation upon addition of the mixture xanthine/xanthine oxydase in the bath (5). Here, we tested if this stimulation is preserved in hTASK2-transfected mammalian cells. Addition of the xanthine/xanthine oxydase increased the outward current 2.5-fold. Representative current traces are depicted in Fig. S3.

COS-7 cells were maintained in Dulbecco's modified Eagle media supplemented with 10% FBS, 100 U/mL streptomycine, and 100 U/mL penicillin at 37 °C in a humidified 5% CO_2 atmosphere. COS-7 Cells were transiently transfected by the DEAE-Dextran precipitate method using 1 μg human TASK2 plasmid per 35-mm culture dish. Currents were recorded 24 h after transfection. Recordings were conducted in the whole-cell configuration at room temperature (~22 °C) using an EPC 10 amplifier (HEKA Electronic) The pipette solution contained (in mM): 150 KCl, 0.5 $MgCl_2$, 5 EGTA, and 10 Hepes, pH 7.3. The bathing media was (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 Hepes, pH 7.3. Pipette resistance was 1.5–2 MΩ. Membrane currents were elicited by 500-ms depolarizations to +40 mV from a holding potential of –80 mV every 15 s. Only cells with series resistance less than 5 MΩ were used for analysis. Data acquisition and analysis were performed using Patchmaster and Fitmaster (HEKA Electronic) and IgorPro (WaveMetrics) softwares. A mixture of 50 μM xanthine and 50 mU/mL xanthine oxidase was added to the bathing media.

1. Brazier SP, Mason HS, Bateson AN, Kemp PJ (2005) Cloning of the human TASK-2 (KCNK5) promoter and its regulation by chronic hypoxia. *Biochem Biophys Res Commun* 336:1251–1258.
2. Warth R, et al. (2004) Proximal renal tubular acidosis in TASK2 K+ channel-deficient mice reveals a mechanism for stabilizing bicarbonate transport. *Proc Natl Acad Sci USA* 101:8215–8220.
3. Mironov SL, Langohr K (2007) Modulation of synaptic and channel activities in the respiratory network of the mice by NO/cGMP signalling pathways. *Brain Res* 1130:73–82.
4. Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol Rev* 87:245–313.
5. Duprat F, Girard C, Jarretou G, Lazdunski M (2005) Pancreatic two P domain K+ channels TALK-1 and TALK-2 are activated by nitric oxide and reactive oxygen species. *J Physiol* 562:235–244.

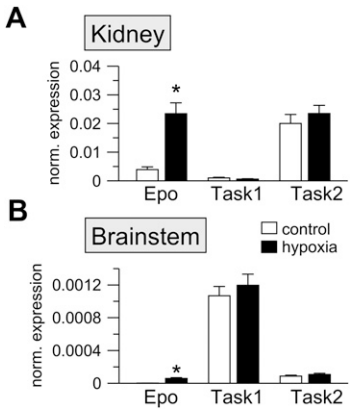

Fig. S1. Mouse Task2 is not a hypoxia-regulated gene in vivo. Using real-time PCR, the effect of 24 h hypoxia exposure of mice (10% O_2) on erythropoietin (Epo), Task1, and Task2 mRNAs was investigated (n = 8, each group). (*A*) In the kidney, mRNA of Epo was induced by hypoxia; the mRNAs for Task1 and Task2 were not changed. (*B*) In mouse brainstem, only the mRNA of Epo was changed by hypoxia pretreatment of the animals. Expression of the target genes was normalized to β-actin expression. All animals were 3- to 6-month-old male mice. An asterisk indicates significant difference from the value of control animals.

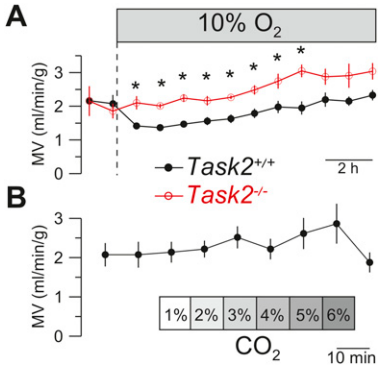

Fig. S2. Effect of metabolic acidosis on hypoxia and CO_2 sensitivity. (*A*) The effect of long-term hypoxia in mice with mild metabolic acidosis was measured by plethysmography. Drinking water of *Task2*$^{+/+}$ (n = 8) and *Task2*$^{-/-}$ mice (n = 4) was replaced by NH_4Cl (50 mM) 12 h before the measurement. Hypoxic depression of the minute volume was still present in acidotic *Task2*$^{+/+}$ but not in *Task2*$^{-/-}$ mice. (*B*) The effect of stepwise increases in CO_2 on minute volume was measured in acidotic *Task2*$^{+/+}$ animals under hyperoxic conditions (n = 7). The effect of CO_2 was attenuated compared with control conditions shown in Fig. 3 (mainly because of an increased minute volume in the absence of CO_2), but acidotic wild-type animals did not show a strong response at 1% and 2% CO_2. All animals were 3- to 6-month-old male mice. An asterisk indicates statistical significance.

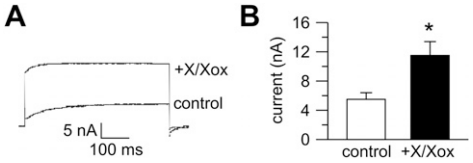

Fig. S3. ROS activate hTASK2 channels. (*A*) Typical traces of an hTASK2-transfected COS-7 cell. Xanthine/xanthine oxidase was used to induce the generation of reactive oxygen species. Whole-cell currents were recorded during a voltage step from −80 mV to +40 mV. Application of xanthine plus xanthine oxidase (+X/Xox) increased the current substantially compared to control conditions (control). (*B*) Summary of similar experiments (n = 20, paired experiments). The asterisk indicates statistical significance.

Table S1. Effect of anoxia, acidosis, and alkalosis on respiratory parameters in in vitro *en bloc* preparation of neonatal mouse brainstem

	Control	Anoxia	Acidosis	Alkalosis
		Duration of inspiratory phase (TI)		
Task2$^{+/+}$	0.383 ± 0.07	0.720 ± 0.14*	0.319 ± 0.04	0.335 ± 0.10
Task2$^{+/-}$	0.386 ± 0.06	0.574 ± 0.11*	0.358 ± 0.04	0.322 ± 0.09
Task2$^{-/-}$	0.255 ± 0.03	0.473 ± 0.09*	0.269 ± 0.05	0.282 ± 0.05
		Amplitude of inspiratory bursts (% of control)		
Task2$^{+/+}$	100	98.1 ± 10.5	137.9 ± 9.2*	108.0 ± 13.3
Task2$^{+/-}$	100	91.8 ± 13.4	127.1 ± 10.2*	102.6 ± 16.5
Task2$^{-/-}$	100	117.1 ± 15.5	119.9 ± 14.2*	95.7 ± 15.0
		Surface of inspiratory bursts (% of control)		
Task2$^{+/+}$	100	136.0 ± 28.5	161.4 ± 30.3	130.5 ± 22.2
Task2$^{+/-}$	100	148.2 ± 23.8	118.0 ± 19.8	157.4 ± 46.8
Task2$^{-/-}$	100	174.7 ± 32.4	148.9 ± 49.4	175.7 ± 51.4

The values were obtained during the experiments shown in Fig. 5*E* (no. of experiments: Task2$^{+/+}$: n = 10; Task2$^{+/-}$: n = 8; Task2$^{-/-}$: n = 7).
*This value was significantly different from the corresponding control value.