Carotid body chemosensory responses in mice deficient of TASK channels

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Background K$^+$ channels of the TASK family are believed to participate in sensory transduction by chemoreceptor (glomus) cells of the carotid body (CB). However, studies on the systemic CB-mediated ventilatory response to hypoxia and hypercapnia in TASK1- and/or TASK3-deficient mice have yielded conflicting results. We have characterized the glomus cell phenotype of TASK-null mice and studied the responses of individual cells to hypoxia and other chemical stimuli. CB morphology and glomus cell size were normal in wild-type as well as in TASK1$^{-/-}$ or double TASK1/3$^{-/-}$ mice. Patch-clamped TASK1/3-null glomus cells had significantly higher membrane resistance and less hyperpolarized resting potential than their wild-type counterpart. These electrical parameters were practically normal in TASK1$^{-/-}$ cells. Sensitivity of background currents to changes of extracellular pH was drastically diminished in TASK1/3-null cells. In contrast with these observations, responsiveness to hypoxia or hypercapnia of either TASK1$^{-/-}$ or double TASK1/3$^{-/-}$ cells, as estimated by the amperometric measurement of catecholamine release, was apparently normal. TASK1/3 knockout cells showed an enhanced secretory rate in basal (normoxic) conditions compatible with their increased excitability. Responsiveness to hypoxia of TASK1/3-null cells was maintained after pharmacological blockade of maxi-K$^+$ channels. These data in the TASK-null mouse model indicate that TASK3 channels contribute to the background K$^+$ current in glomus cells and to their sensitivity to external pH. They also suggest that, although TASK1 channels might be dispensable for O$_2$/CO$_2$ sensing in mouse CB cells, TASK3 channels (or TASK1/3 heteromers) could mediate hypoxic depolarization of normal glomus cells. The ability of TASK1/3$^{-/-}$ glomus cells to maintain a powerful response to hypoxia even after blockade of maxi-K$^+$ channels, suggests the existence of multiple sensor and/or effector mechanisms, which could confer upon the cells a high adaptability to maintain their chemosensory function.

INTRODUCTION

Oxygen-regulated K$^+$ channels, initially described in the rabbit carotid body (CB) glomus cell (López-Barneo et al., 1988; Ganfornina and López-Barneo, 1991), are believed to play a fundamental role in chemosensory transduction. It is broadly accepted that reduction of glomus cell K$^+$ conductance in hypoxemia is the major event leading to depolarization and Ca$^{2+}$ channel opening, rise of cytosolic [Ca$^{2+}$], and transmitter release. These transmitters stimulate afferent nerve fibers acting on brainstem respiratory neurons to evoke hyperventilation (López-Barneo et al., 1993; Buckler and Vaughan-Jones, 1994; Ureña et al., 1994; Montoro et al., 1996; for recent reviews see Prabhakar, 2006; López-Barneo et al., 2008). Different functional subtypes of O$_2$-regulated K$^+$ channels have been reported in glomus cells from several mammalian species (Peers, 1990; Stea and Nurse, 1991; Ganfornina and López-Barneo, 1992; Wyatt and Peers, 1995; Buckler, 1997; Pérez-García et al., 2004) as well as in other neurosecretory cell classes acutely responding to hypoxia (for review see López-Barneo et al., 2001; Nurse et al., 2006).

Although the understanding of the cellular bases of CB chemotransduction has advanced considerably, the precise molecular nature of the O$_2$ sensor(s) and the effector K$^+$ channel(s) is unknown (see Kemp, 2006). Progress in this field is hampered by methodological limitations derived from the gaseous nature of the stimulus and the delicacy of the O$_2$-sensing apparatus, which can be altered during cell dissociation (Ortega-Sáenz et al., 2007). Additionally, the small size of the CB has precluded large-scale biochemical analyses. These limitations can be partially overcome by the use of genetically modified mice, in which the functional consequences of targeted molecular ablation can be unambiguously demonstrated (e.g., Ortega-Sáenz et al., 2006; Mulkey et al., 2007). To this end, we developed...
the mouse CB thin slice preparation, where reproducible responses of glomus cells to chemosensory stimuli can be routinely obtained (Piruat et al., 2004; Ortega-Sáenz et al., 2007).

Here, we have evaluated the chemosensitivity of CB glomus cells from mice deficient of TASK channels. These belong to the tandem pore domain (K2P) family of channels and contribute to the leak or background K⁺ conductance in a broad variety of cells. TASK1 (Kcnk3 or K2P3.1) and TASK3 (Kcnk9 or K2P9.1), the relevant members of the TASK channel class (Duprat et al., 1997; Kim et al., 2000; Rajan et al., 2000), can form heteromers (Czirják and Enyedi, 2002) and have been proposed to be involved in peripheral and central chemoreception (Bayliss et al., 2001; Feldman et al., 2003; Mulkey et al., 2004). Recombinant TASK1 channel activity is reduced upon exposure to low O₂ tension (Kemp et al., 2004; Lee et al., 2006; however, for contrasting results see Johnson et al., 2004), and these channels appear to mediate the hypoxic depolarization of cerebellar granule cells (Plant et al., 2002). In rat CB glomus cells, an O₂-sensitive TASK-like standing K⁺ current with weak outward rectification (in physiological asymmetrical K⁺) and blocked by extracellular Ba²⁺ but resistant to the classical K⁺ channel blockers TEA and 4-aminopyridine (4-AP), has been reported (Buckler, 1997). The CB standing K⁺ current shares other pharmacological properties, such as activation by the volatile anesthetic halothane and inhibition by anandamide, with currents mediated by TASK1 channels (Buckler et al., 2000). Moreover, background single K⁺ channel current activity in glomus cells shows flickering kinetics and slope conductance compatible with those reported for recombinant, heterologously expressed TASK1 channels (Williams and Buckler, 2004). Therefore, a popular view is that TASK1 channels may be fundamental for CB O₂ sensing (Duprat et al., 2007). However, a detailed recent study by Kim et al. (2009) has shown that heteromeric TASK1/TASK3 are the major O₂-sensitive background K⁺ channels in rat CB glomus cells.

In recent years, TASK1- and/or TASK3-deficient mice have been independently generated in two laboratories that reported the animals to be healthy and with normal lifespan (Aller et al., 2005; Brickley et al., 2007; Mulkey et al., 2007). The absence of TASK1 and/or TASK3 results only in a minor phenotype in central neurons, despite the loss of acid sensitivity in some neuronal groups and the complete disappearance of halothane effect on membrane currents or conductance. In some mice strains, invalidation of TASK1 channels disrupts adrenal gland zonation and produces hyperaldosteronism (Heitzmann et al., 2008). However, the impact of TASK deficiency on peripheral chemoreception is a subject of controversy. Although one group has reported normal ventilatory responses to hypoxia and hypercapnia in the double TASK1 and TASK3 (TASK1/3⁻/⁻) knockout animals (Mulkey et al., 2007), another laboratory has observed in TASK1, but not in TASK3, knockout mice decreased ventilation and afferent sinus nerve discharges in response to hypoxia and hypercapnia (Trapp et al., 2008). Herein, we report the basic electrophysiological properties and intrinsic chemosensory activity of individual glomus cells from TASK-deficient animals. We show that glomus cells from TASK1-null animals appear to be normal, but TASK1/3 knockout cells exhibit characteristic electrophysiological alterations and decreased sensitivity to external pH. Nonetheless, the secretory responses of TASK1/3⁻/⁻ cells to hypoxia as well as other chemosensory stimuli remain essentially unaltered. The implications of these findings for CB O₂ sensing are discussed.

MATERIALS AND METHODS

Animals
For the experiments, we used young adult (2-6-month-old) TASK-null mice (either TASK1⁻/⁻, TASK3⁻/⁻, or double TASK1/3⁻/⁻) and the corresponding wild-type littermates provided by M. Aller (Instituto de Neurociencias, Alicante, Spain). The nonfunctional alleles were generated as described in detail previously (Aller et al., 2005; Brickley et al., 2007). Mice were genotyped as described previously (Aller et al., 2005; Brickley et al., 2007). Animal care and experimentation were performed according to the institutional animal care committee guidelines.

RNA analysis
Four animals for every genotype were killed by sodium pentobarbital overdose (intraperitoneally [i.p.]), and the CBs were dissected, pooled, and stored in liquid nitrogen. mRNA was extracted using Dynabeads mRNA DIRECT micro kit (Invitrogen). First-strand cDNA was synthesized from total mRNA extraction using the Superscript first-strand synthesis system for reverse transcription (RT)-PCR (Invitrogen). PCR amplifications of TASK1, TASK3, and GAPDH mRNAs were performed using the following primers: TASK1 (Kcnk3; 515 bp): 5'-CACCCTCACACTACACATG-3' and 5'-TGCTTCGATCAGCCATTTC-3'; TASK3 (Kcnk9; 413 bp): 5'-ATGAAGTGCAGGAGAGAAAAC-3' and 5'-AGGAGGCCATGGAAGAAAAAG-3'; and GAPDH (255 bp): 5'-CAGAATTGGAGGTCGCTCTG-3' and 5'-TTTGTAGTGTTCCGGGTCTG-3'. For quantitative RT-PCR analysis, four groups of three young adult double TASK1/3⁻/⁻ or control mice were killed by pentobarbital overdose (i.p.), and the CBs were processed as described above. Real-time PCR was performed in an ABI Prism 7500 Sequence Detection System (Applied Biosystems) using SYBR Green PCR Master mix (Applied Biosystems) and the thermocycler conditions recommended by the manufacturer. Each sample was analyzed for cyclophilin to normalize for RNA input amounts and to perform relative quantifications. To normalize mRNA levels in knockout mice to those in control samples, we calculated an average cycle threshold of the control samples and processed all the samples in the experiment relative to this average cycle threshold. Primers were designed using the computer program Primer Express (Applied Biosystems). The following primers were used: maxi-K⁺ channel α subunit (Kcnm2; 76 bp): 5'-CATGCGTCTCCACATGCTCTC-3' and 5'-GCCAGAAACCCAGGTTATGCTG-3'; TASK5 (Kcnk15; 55 bp): 5'-GCTGTTTCATGTGCTTCATC-3' and 5'-ACGAGTCGCGAAGCT-3'; and cyclophilin A (Ppia; 75 bp): 5'-GCACTGCTGCGAAGCTC-3' and 5'-GCCAGAAACCCAGGTTATGCTG-3'. Melting curve analysis showed a single sharp peak with the expected Tm for all samples.

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Immunocytochemistry and morphological studies
For every genotype, three animals were killed by sodium pentobarbital overdose (i.p.), and the carotid bifurcations were dissected, washed with PBS, fixed 2 h at 4°C in 4% paraformaldehyde, and equilibrated for 12 h in a 30% sucrose solution. Bifurcations were included in OCT (optimal cutting temperature; Tissue Tek; Sakura) and snap-frozen by quenching in dry ice. 10-µm thick slices were cut with a cryostat. Sections were stained with the anti-tyrosine hydroxylase (TH) polyclonal antibody (1:1,000; Pel-Freez Biologicals). The Envision+ kit (Dako) was used for immunohistochemistry according to the manufacturer’s recommended protocol. The signal was developed with DAB (Dako). Images were acquired under a microscope (BX-61; Olympus). Estimation of CB volume was performed across the entire CB parenchyma using the CAST Grid System. A Cavalieri size of 912.7 µm² was used.

Patch clamp recordings
Macroscopic currents were recorded from dispersed mouse glomus cells using either the perforated patch or the whole cell configurations of the patch clamp technique as adapted to our laboratory (Muñoz-Cabello et al., 2005; García-Fernández et al., 2007). Preparation of dispersed mouse CB cells was performed as described previously (Piruat et al., 2004; Ortega-Sáenz et al., 2006). Patch electrodes (1.5–2.5 MΩ) were pulled from capillary glass tubes (1.5–1.6 mm OD; Kimax; Kimble Products), fire polished on a microforge MF-830 (Narishige), and coated with silicone elastomer (Sylgard 184; Corning) to decrease capacitance. Voltage clamp recordings were obtained with an EPC-8 patch clamp amplifier (HEKA) using standard voltage clamp protocols designed with Pulse software (HEKA). Unless otherwise noted, holding potential was ~80 mV. Data were filtered at 10 kHz, digitized at a sampling interval of 20 µs with an ITC-16 A/D converter (HEKA), and stored on a Macintosh computer. Offline analysis of data was performed using custom software and Pulse Fit (HEKA). All experiments were conducted at room temperature, 23–26°C. Experiments designed to estimate the cell’s resting potential and input resistance, as well as the pH dependence of the background K⁺ currents, were made using perforated patches with amphotericin B in the pipette solution. This solution also contained (in mM): 70 K₂SO₄, 30 KCl, 2 MgCl₂, 1 EGTA, and 10 HEPES, pH 7.2. The standard bath solution contained (in mM): 140 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, 2.5 CaCl₂, and 4 MgCl₂, pH 7.4. For the pH experiments, the external solutions contained (in mM): 3 KCl, 118 NaCl, 1 MgCl₂, 1.5 CaCl₂, 25 HEPES, and 10 glucose, with the pH adjusted to the desired level using NaOH or HCl. We also added 10 mM TEA and 5 mM 4-AP to the solution to block voltage-dependent K⁺ channels. Estimated values of resting potential are given after correction for junction potentials. Macroscopic Ca²⁺, Na⁺, and K⁺ currents were recorded in dialyzed glomus cells. The solutions used for the recording of whole cell Na⁺ and Ca²⁺ currents contained (in mM): external: 140 NaCl, 9 BaCl₂, 1 CaCl₂, 10 HEPES, and 10 glucose; pH 7.4 and osmolality 300 mosm/kg; and internal: 110 CaCl₂, 30 CsF, 10 EGTA, 10 HEPES, and 4 ATP-Mg; pH 7.2 and osmolality 285 mosm/kg. The solutions used for the recording of whole cell K⁺ currents contained (in mM): external: 140 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, 2.5 CaCl₂, and 4 MgCl₂, pH 7.4; and internal: 80 potassium glutamate, 50 KCl, 1 MgCl₂, 10 HEPES, 4 MgATP, and 5 EGTA, pH 7.2.

Amperometric recording of single-cell catecholamine secretion in slices
CB slices were used because the most reproducible single–glomus cell responses to hypoxia are obtained in this preparation (Pardal et al., 2000). Mice CB dissection, slicing, and culture, as well as the measurement of catecholamine secretion, were performed following the same procedures described previously (Ortega-Sáenz et al., 2003, 2006). CBs were resected, cleaned of connective tissue, and included in agarose. After mounting the piece on the stage of a vibratome, 150-µm thick slices were cut, placed in a Petri dish with culture medium (Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, 1% penicillin/streptomycin, 1% l-glutamine, and 84 U of insulin per ml), and maintained at 37°C in a 5% CO₂ incubator for 24–48 h. Slices were transferred to a recording chamber and continuously perfused with a solution containing (in mM): 117 NaCl, 4.5 KCl, 23 NaHCO₃, 1 MgCl₂, 2.5 CaCl₂, 5 glucose, and 5 sucrose. The osmolality of the solution was ~280 mosm/kg. The “normoxic” solution was bubbled with a gas mixture of 5% CO₂, 20% O₂, and 75% N₂ (O₂ tension, ~145 mm Hg).

Figure 1. Molecular and histological characterization of TASK1-and double TASK1/3–deficient mice. (A) RT-PCR analysis showing the absence of Task1 or Task3 mRNA expression in the CB of TASK1- or TASK1/3-null mice. (B) CB anatomy in young TASK1- or TASK1/3-deficient animals. Representative sections of control (wild type; left), TASK1−/− (middle), and TASK1/3−/− (right) carotid bodies. CB glomus cells are stained with an antibody against TH. IC, internal carotid artery. (C) Graph representing the CB volume occupied by TH-positive cells. Data are n = 3 for each experimental condition.
The “hypoxic” solution was bubbled with 5% CO₂ and 95% N₂ to reach an O₂ tension in the chamber of ~15 mm Hg. To perform dose–response curves, the solutions were also bubbled with 12 and 6% O₂, keeping CO₂ at 5%. When these solutions were used, the approximate values of O₂ tension in the chamber were, respectively, 90 and 50 mm Hg. Experiments of secretion induced by hypercapnia were done with the same control solution bubbled with 10% CO₂, 20% O₂, and 70% N₂ or 20% CO₂, 20% O₂, and 60% N₂ (10 or 20% hypercapnia, respectively). In these conditions, extracellular pH decreased from ~7.3 in 5% CO₂ to 7.1 in 10% CO₂ or 6.8 in 20% CO₂. All the experiments were made at a temperature in the chamber of ~36°C. Secretory events were recorded with a polarized (+750 mV) 10-µm carbon fiber electrode positioned near a cell under visual control and connected to the current-to-voltage converter of an EPC-8 patch clamp amplifier. Amperometric currents were filtered at 100 Hz and stored on a computer. The cumulative secretion signal was obtained by the sum of the time integral of successive amperometric events (Pardal and López-Barneo, 2002). Secretion rate (in either femto- or pico-coulombs/min) was calculated as the amount of charge transferred to the recording electrode during a given time period.

**Statistical analysis**

Unless otherwise specified, data are expressed as mean ± SE, with the number (n) of experiments indicated. Statistical analysis was performed by unpaired Student’s t test. A value of P < 0.05 was considered as statistically significant.

**Online supplemental material**

Confocal fluorimetric recordings of intracellular Ca²⁺ concentration in dispersed mouse glomus cells are shown in Fig. S1. The data indicate that in both wild-type and TASK1/3–null glomus cells, hypoxia induces an increase of intracellular Ca²⁺ concentration. In Fig. S2, we show in dispersed chromaffin cells the inhibition of...
experiments were performed on glomus cells from the TASK1/3 double knockout, although, when necessary, experiments were also done on single TASK1-null cells because these last channels are those proposed to participate more specifically in CB O₂ sensing (see Duprat et al., 2007; Trapp et al., 2008). In our experimental conditions, the estimated resting membrane potential of dispersed wild-type cells recorded with the perforated patch technique was $-57 \pm 1.2$ mV ($n = 24$); this value was only slightly changed in TASK1-deficient cells ($-54.5 \pm 1.8$ mV; $n = 29$) but decreased significantly (to $-51.5 \pm 0.8$ mV; $n = 33$; $P < 0.05$) in TASK1/3-null cells. Glomus cells from TASK1/3 knockout mice also had a statistically significant increase of input resistance compared with controls (Table I).

Recombinant TASK1 and TASK3 channels are highly sensitive to extracellular pH due to a proton-sensing histidine residue located at the external mouth of the channels (Rajan et al., 2000). Based on pharmacological experiments, it has been proposed that TASK1 channels contribute to pH sensing in glomus cells (Buckler et al., 2000). In cells bathed in external solution containing TEA and 4-AP to minimize the ion fluxes through voltage-dependent K⁺ channels, currents evoked by depolarizing ramps were increased by pH alcalinization (switching from pH 6.3 to 8.2) and, as expected, this effect was small or even negligible in TASK1/3-null cells (Fig. 2, A and B). Upon exposure to pH 8.2, outward K⁺ current density at $-20$ mV in wild-type cells was on average fivefold larger than in cells from TASK1/3-deficient animals (Fig. 2 B and Table I). In parallel with the effect on K⁺ current density, ablation of the TASK1/3 channel

**RESULTS**

**Morphology of TASK-null carotid bodies**

In wild-type animals, we confirmed by PCR the expression of TASK1 and TASK3 mRNAs in the CB and their complete disappearance in TASK-deficient animals (Fig. 1 A). Immunocytochemical analyses with anti-TH antibodies demonstrated in all the animal types that the CBs were normal and appeared organized in the characteristic clusters (glomeruli) of TH-positive glomus cells (Pardal et al., 2007) (Fig. 1 B). The volume occupied by the CB parenchyma was similar in TASK1 or TASK1 and TASK3 double (TASK1/3) knockout mice compared with wild-type littermates (Fig. 1 C). The size of individual CB glomus cells, as indicated by the value of total capacitance measured in perforated patch-clamped cells, was also similar in the three animal strains (see Table I).

**Electrophysiological parameters and pH sensitivity of TASK-deficient glomus cells**

TASK1 and TASK3 channels are expressed in numerous neural and non-neural tissues, as well as in the CB. These channels are open over a broad range of membrane voltages and contribute to set the cell’s resting potential and membrane resistance. Hence, we sought to see whether the absence of TASK channels resulted in modifications in the electrophysiological parameters of glomus cells. For the sake of simplicity, some of these experiments were performed on glomus cells from the TASK1/3 double knockout, although, when necessary, experiments were also done on single TASK1-null cells because these last channels are those proposed to participate more specifically in CB O₂ sensing (see Duprat et al., 2007; Trapp et al., 2008). In our experimental conditions, the estimated resting membrane potential of dispersed wild-type cells recorded with the perforated patch technique was $-57 \pm 1.2$ mV ($n = 24$); this value was only slightly changed in TASK1-deficient cells ($-54.5 \pm 1.8$ mV; $n = 29$) but decreased significantly (to $-51.5 \pm 0.8$ mV; $n = 33$; $P < 0.05$) in TASK1/3-null cells. Glomus cells from TASK1/3 knockout mice also had a statistically significant increase of input resistance compared with controls (Table I).

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genes also abolished the decrease of membrane resistance induced by alcalinization (Fig. 2 C). Collectively, these data suggest that the lack of TASK channels in CB glomus cells (particularly TASK3) does indeed result in consistent alterations of their electrophysiological parameters, as well as the responsiveness to changes in extracellular pH.

To further investigate the TASK-null CB phenotype, we measured the density of voltage-dependent K+, Na+, and Ca2+ currents in wild-type and TASK1 or TASK1/3 knockout glomus cells. Large voltage-dependent outward K+ currents were recorded in the three glomus cell types (Fig. 3 A). The amplitude of the current was unchanged in TASK1−/− cells, but a clear reduction of K+ current density (∼50%) was observed in TASK1/3−/− preparations (Fig. 3 B and Table I). We have tested by quantitative RT-PCR whether the mRNA expression of other K+ channel genes is altered in TASK1/3−/− CB cells. TASK5 channels, the closest relative to TASK1 and TASK3 within the TASK family (Duprat et al., 2007), were not significantly expressed in the CB tissue. The maxi-K+ channel α subunit, functionally expressed in mouse CB (Yamaguchi et al., 2004; Ortega-Sáenz et al., 2006), appeared to be down-regulated in TASK1/3−/− animals (Fig. 3 C).

Dispersed mouse glomus cells exhibited relatively large Na+ and/or Ca2+ inward currents (Fig. 4 A). The current–voltage relation of the Na+ current was unaltered in TASK-deficient cells, and TASK1−/− cells also had Ca2+ currents of normal amplitude (Table I and Fig. 4, B and C). However, in TASK1/3−/− cells, the peak Ca2+ current density decreased to ∼50% of control values, although the voltage dependence of the current remained unchanged (Table I and Fig. 4 C). As their rabbit counterparts (Ureña et al., 1989), mouse glomus cells have two well-represented distinct populations of Ca2+ channels that were easily separated by their deactivations.
kinetics (at $-70 \text{ mV}$: $\tau_{\text{fast}} = 0.123 \pm 0.009 \text{ ms}$ and $\tau_{\text{slow}} = 1.60 \pm 0.12 \text{ ms}$; $n = 25$; Fig. 4 D). These correspond to high threshold, or fast deactivating, and low threshold, or slowly deactivating, Ca$^{2+}$ channels, which in neuro-secretory cells can contribute to transmitter release (see Carabelli et al., 2007; Levitsky and López-Barneo, 2009). The deactivation time constants of these channel types were also indistinguishable between wild-type and TASK-null cells (TASK1$^+/-$: $\tau_{\text{fast}} = 0.093 \pm 0.006 \text{ ms}$ and $\tau_{\text{slow}} = 1.47 \pm 0.09 \text{ ms}$; $n = 12$; TASK1/3$^+/-$: $\tau_{\text{fast}} = 0.106 \pm 0.005 \text{ ms}$ and $\tau_{\text{slow}} = 1.66 \pm 0.09 \text{ ms}$; $n = 34$). The decrease of Ca$^{2+}$ current density observed in TASK1/3$^+/-$ glomus cells was mainly due to decrease of the high voltage-activated current, the most predominant component in glomus cells (Fig. 4 E). Nevertheless, the density of the low voltage-activated current was also significantly reduced in the TASK1/3 knockout cells (Fig. 4 F).

Chemosensory responses to hypoxia of glomus cells from TASK-null mice
Responsiveness of CB glomus cells to hypoxia in TASK-deficient mice was studied using thin CB slices, where the intrinsic O$_2$ sensitivity of intact glomus cells can be evaluated separately from the other steps along the chemosensory pathway involved in the hypoxic ventilatory responses (Piruat et al., 2004; Ortega-Sáenz et al., 2006). Representative recordings of catecholamine release from individual glomus cells subjected to low O$_2$ tension (PO$_2$, $\sim 15 \text{ mm Hg}$) are illustrated in Fig. 5 A, and a quantitative summary of the secretion rate in normoxic and hypoxic conditions is shown in Fig. 5 (B and C). Secretion rate induced by hypoxia in wild-type glomus cells ($5,133 \pm 1,010 \text{ fC/min}$; $n = 7$) was similar to the values observed in cells deficient of either TASK1 ($5,175 \pm 719 \text{ fC/min}$; $n = 12$) or TASK1/3 ($5,939 \pm 1,595 \text{ fC/min}$; $n = 5$) channels (Fig. 5 B). Basal secretion in normoxic conditions was, however, over twofold higher in TASK1/3-null cells than in the two other glomus cell types (Fig. 5 C). As the Ca$^{2+}$ channel density was reduced in TASK1/3$^+/-$ cells, we also tested that, similar to other rodent glomus cells (Pardal et al., 2000; Piruat et al., 2004), the secretory response to hypoxia was abolished by blockade of Ca$^{2+}$ channels with Cd$^{2+}$ (Fig. 5 A, inset, bottom), thus suggesting that it was triggered by transmembrane Ca$^{2+}$ influx. Preliminary experiments performed on dispersed glomus cells also indicate that hypoxia induces similar rises of cytosolic [Ca$^{2+}$] in wild-type and TASK1/3-null cells (Fig. S1).

Sensitivity to hypoxia of TASK-null glomus cells was further studied by dose–response experiments in which mouse CB slices were exposed to various O$_2$ levels. Glomus cells showed a graded response upon exposure to progressively higher hypoxia with a characteristic hyperbolic correlation that was practically similar in all the animal strains studied (wild type, TASK1 null, and TASK1/3 null). These dose–response relationships at the cellular level are almost similar to the correlation between arterial PO$_2$ and the afferent discharges of the CB sinus nerve recorded in vivo or in the explanted CB in vitro (Fig. 6, A and B).
studied whether in the mutant animals the \(O_2\)-sensitive maxi-K\(^+\) current was necessary to compensate for the lack of TASK channels. In these experiments, maxi-K\(^+\) channels were blocked with paxilline, a broadly used selective antagonist (Gribkoff et al., 1996; Sheehan et al., 2009) that in our experimental conditions fully inhibited maxi-K\(^+\) channels (Fig. S2). In the presence of high concentrations of paxilline, the secretory response to hypoxia of TASK1/3-deficient cells remained unaltered (Fig. 7 B).

**Figure 6.** Responsiveness of CB glomus cells to different levels of hypoxia in wild-type, TASK1-, and double TASK1/3-null mice. (A) Amperometric recordings of catecholamine secretion induced by different levels of \(O_2\) tension in CB glomus cells from the indicated mouse strains (control, 21% \(O_2\), 145 mm Hg; 12% \(O_2\), 90 mm Hg; 6% \(O_2\), 50 mm Hg; hypoxia, 15 mm Hg). (B) Dose-response curves estimated from glomus cells of the various mouse strains exposed to solutions equilibrated with various levels of \(O_2\) (150, 90, 50, and 15 mm Hg). Data are from three to seven experiments. The average secretion rate values in wild-type, TASK1\(^-/-\), and TASK1/3\(^-/-\) glomus cells at each \(O_2\) tension were not significantly different (\(P > 0.05\)).

To gain further insight into the role of TASK channels in CB \(O_2\) sensing, we performed amperometric experiments in normal (wild-type) mouse glomus cells exposed to acidic pH to minimize the activity of pH-sensitive background channels. Low pH, which presumably inhibits TASK channels (see Fig. 2), induced secretory activity in glomus cells but did not prevent a full response upon subsequent exposure to hypoxia. Indeed, low pH appeared to potentiate the effect of hypoxia (Fig. 7 A). Although TASK1/3\(^-/-\) glomus cells showed down-regulation of both macroscopic K\(^+\) current amplitude (Fig. 3 B) and maxi-K\(^+\) channel mRNA expression (Fig. 3 C), we

**DISCUSSION**

**Morphological and electrophysiological properties of TASK1- and TASK1/3-null glomus cells**

In this paper, we show that TASK1- or TASK1/3-null mice have morphologically normal CB, with TH-positive glomus cells arranged in clusters (glomeruli) typical of the CB parenchyma (Pardal et al., 2007). TASK1-deficient glomus cells had normal passive electrophysiological parameters. In contrast, glomus cells lacking both TASK1 and TASK3 channels showed a clear electrophysiological phenotype characterized by an increase of membrane resistance and cell depolarization. These observations suggest that TASK3 channels (or heteromers of TASK1 and TASK3 channels) contribute to set the resting potential of normal mouse glomus cells (see Kim et al., 2009).

Background K\(^+\) currents recorded from TASK1/3-null glomus cells were less sensitive to changes in extracellular pH than those in control cells. This finding fits well with the proposal that TASK-like channels participate in extracellular acid sensing in the CB (Buckler et al., 2000; for discussion on CB acid sensing see López-López...
significant population of low threshold T-type Ca\textsuperscript{2+} channels that, as described in neonatal or hypoxic adult chromaffin cells (Carabelli et al., 2007; Levitsky and López-Barneo, 2009), could be activated by small membrane depolarizations to induce exocytosis. Indeed, we have observed an increased basal secretion rate in TASK1/3-null cells (see below), which is compatible with the sustained depolarization and increased excitability of this cell type.

Voltage-dependent (Na\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+}) current densities were normal in TASK1 knockout glomus cells; however, K\textsuperscript{+} and Ca\textsuperscript{2+} currents were markedly reduced in TASK1/3-deficient cells. These observations further support the view that although TASK1 channels might be dispensable, TASK3 channels (either as homomers or TASK1/TASK3 heteromers) are absolutely required for the maintenance of the physiological phenotype of glomus cells. The decrease of Ca\textsuperscript{2+} and K\textsuperscript{+} current density in TASK1/3 knockout cells could be the result of an electrophysiological remodeling induced by the persistent depolarization of these cells. In this regard, it is known that L-type Ca\textsuperscript{2+} channel α subunit is down-regulated by chronic depolarization in PC12 and smooth muscle cells.

Figure 7. Maintenance of the secretory response to hypoxia in wild-type and TASK1/3\textsuperscript{-/-} glomus cells after blockade of different potassium channels. (A) Amperometric recording and corresponding cumulative secretion signal (in pC) of catecholamine release induced by hypoxia (15 mm Hg) in wild-type CB glomus cells before and after blockade of TASK channels with acidic pH. The composition of the control solution (pH 7.4) is indicated in Materials and methods and contained 23 mM HCO\textsubscript{3}\textsuperscript{-}. In the acidic (pH 6.5) solution, this was replaced with 10 mM HCO\textsubscript{3}\textsuperscript{-} plus 12 mM NaH\textsubscript{2}PO\textsubscript{4} and 1 mM Na\textsubscript{2}HPO\textsubscript{4}. Secretion rate during hypoxia and acidic hypoxia were 3,694 and 5,888 fC/min, respectively. (B) Secretory response to hypoxia and corresponding cumulative secretion signal (in pC) in TASK1/3-null mice glomus cells in the presence of the maxi-K\textsuperscript{+} channel blocker paxilline (500 nM). Secretion rate during hypoxia was 3,193 fC/min and changed to 4,095 fC/min in the presence of paxilline.
Glomus cell $O_2/CO_2$ sensing in TASK1/3-null mice

(Yamaguchi et al., 2004; Ortega-Sáenz et al., 2006), was down-regulated in TASK1/3-null animals.

Secretory activity and responsiveness to hypoxia/hypercapnia of TASK-null glomus cells

TASK1 deficiency did not cause any appreciable alteration in the secretory activity of glomus cells. However, we systematically observed an increase in the basal secretion rate of TASK1/3-null glomus cells, which is compatible with the enhanced excitability characteristic of cells from this animal strain. Sensitivity of either TASK1- or TASK1/3-deficient glomus cells to modifications of environmental $O_2$ or $CO_2$ tension was indistinguishable with respect to wild-type glomus cells. In fact, $O_2$ or $CO_2$ dose-response curves were quite similar among the CB cells of the various genetically modified animals studied. Moreover, after the exposure of wild-type glomus cells to low pH, which blocked TASK1 and TASK3 channels, responsiveness to hypoxia was maintained or even enhanced. Potentiation of hypoxia by hypoglycemia, a response characteristic of glomus cells (Pardal and López-Barneo, 2002; García-Fernández et al., 2007), was also maintained in TASK1/3-null carotid bodies (see also Guyon et al., 2009).

The observations summarized above are consistent with the lack of electrophysiological phenotype exhibited by TASK1-null cells and further suggest that TASK1 channels, although they could contribute to the formation of TASK1/3 heteromers in normal animals, might not be absolutely required for the maintenance of chemosensory function in mouse glomus cells. However, it is surprising that TASK1/3-deficient cells showed normal secretory responses to hypoxia and hypercapnia, despite the fact that they have major electrophysiological alterations. The most likely explanation is that the increase of TASK1/3 knockout cell excitability (membrane depolarization, increase of input resistance, and decrease of voltage-gated $K^+$ currents) compensates for the reduction of $Ca^{2+}$ current density, thus resulting in cells with apparently normal chemosensory function.

These experimental data obtained from the TASK-deficient model are compatible with the existence in normal mouse glomus cells of $O_2$-sensitive, TASK3-mediated currents similar to those described in the rat (Kim et al., 2009). We have shown here that this current regulates the cell’s resting potential, and therefore its inhibition by hypoxia should contribute to the initiation of the chemosensory response. In cells lacking TASK3 channels (due to genetic ablation or blockade by low pH), responsiveness to hypoxia must be necessarily mediated by other $O_2$-sensitive currents, although the depolarization and enhanced excitability resulting after TASK3 inhibition is possibly what makes the cells exhibit quantitatively normal chemosensory responses.

The behavior of TASK1- or TASK1/3-deficient cells observed in CB slices are in accord with the in vivo study (Feron and Godfraind, 1995). On the other hand, maintained $Ca^{2+}$ influx also seems to inhibit the expression of T-type $Ca^{2+}$ channels in PC12 cells (Del Toro et al., 2003). Although the amplitude of the whole cell $K^+$ current decreased in TASK1/3-null glomus cells, it is possible that other specific $O_2$-sensitive $K^+$ channel subtypes were up-regulated to compensate for the lack of TASK1 and TASK3. A detailed study of the expression of the various $K^+$ channel $\alpha$ subunits in TASK1/3 knockout glomus cells was outside the scope of this work. However, we have found that TASK5, the closest relative to TASK1 and TASK3, was not significantly expressed in CB tissue from either wild-type or TASK1/3-null animals. The mRNA expression of the maxi-$K^+$ $\alpha$ subunit, another $O_2$-regulated $K^+$ channel type expressed in mouse glomus cells

![Figure 8](https://example.com/image.png)

**Figure 8.** Responsiveness of CB glomus cells to hypercapnia in wild-type and double TASK1/3-null mice. (A) Amperometric recordings of catecholamine secretion induced by different levels of $CO_2$ (10 and 20% $CO_2$) tension. (B) Dose-response curves estimated from glomus cells of wild-type and TASK 1/3$^{-/-}$ deficient mice exposed to solutions equilibrated with various levels of $CO_2$ (10 and 20% $CO_2$). Data are from 8–12 experiments. The average secretion rate values in wild-type and TASK1/3$^{-/-}$ glomus cells at each CO2 tension were not significantly different ($P > 0.05$).
either by itself or through the induction of other ion channel types. This last possibility is plausible, as the reverse phenomenon, recruitment of TASK channels in neurons devoid of GABA receptors, has been reported (Millar et al., 2000; Brickley et al., 2001).

Implications of findings in TASK-null cells for CB O₂ and CO₂ sensing

The fact that hypoxia responsiveness (in terms of catecholamine release) remains practically intact in TASK-null glomus cells has, in our view, important implications for the mechanisms of CB O₂ (and CO₂) sensing. We have shown that TASK1/3-null cells manage to show a quantitatively “normal” secretory response to hypoxia, even though they have clear electrophysiological alterations resulting from the absence of TASK3 channels. Moreover, responsiveness to hypoxia was maintained in TASK1/3-deficient cells in which maxi-K⁺ currents were pharmacologically blocked, an observation that confirms previous data showing that blockade of maxi-K⁺ channel with iberiotoxin does not prevent a powerful hypoxic secretory response in mouse and rat glomus cells (Ortega-Sáenz et al., 2006). In fact, maxi-K⁺ channel α subunit mRNA appeared to be down-regulated in TASK1/3-deficient cells. Therefore, in the TASK1/3-null mouse model, suppression of two well-established effectors of hypoxia (TASK and maxi-K⁺ channels) is compensated by other mechanisms to minimize the impact that abolishment of these channels might have on the functional responses of glomus cells. These observations suggest that glomus cells have a high adaptive capability to maintain their chemosensory function, possible due to multiplicity of sensor and/or effector mechanisms.

The discrepancies between our results obtained from CB slices and those of Trapp et al. (2008) using the in vitro whole CB sinus nerve preparation could derive, at least in part, from functional differences in the cellular elements monitored with each experimental procedure. The dose–response relation obtained with the amperometric recordings from cells in CB slices almost perfectly matches the characteristic hyperbolic correlation between arterial O₂ tension and afferent discharges of the CB sinus nerve (see Weir et al., 2005). Nevertheless, chemoreceptor nerve excitation may not be strictly proportional to catecholamine release from glomus cells, as the firing of afferent fibers also depends on postsynaptic mechanisms (e.g., the type and density of ion channels involved in repetitive action potential generation and propagation; e.g., see Faustino and Donnelly, 2006).

Thus, it could be that the lack of TASK1 channels, although leaving the glomus cells’ O₂ and CO₂ sensing mechanisms little affected, alters signal transmission at the chemosensory synapse (see Ortega-Sáenz et al., 2007), either by itself or through the induction of other ion channel types. This last possibility is plausible, as the reverse phenomenon, recruitment of TASK channels in neurons devoid of GABA receptors, has been reported (Millar et al., 2000; Brickley et al., 2001).

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It has traditionally been proposed that, as in other sensory systems, CB glomus cells have specific O₂ sensor molecule(s) selectively associated with some K⁺ channel...
type(s) to regulate glomus cell resting potential and/or action potential firing frequency (López-López et al., 1989; López-Barneo, 1994; Kemp, 2006). However, molecular selectivity in hypoxic chemotransduction is difficult to reconcile with the experimental observations, as O₂ sensitivity is a property shared by various subtypes of K⁺, as well as Na⁺ and Ca²⁺, channels broadly distributed in numerous cell types and animal species (for detailed review see López-Barneo et al., 2001; for recent commentary see Wyatt and Peers, 2009). The results of our current study further suggest that whatever the nature of the O₂ sensor mechanism(s) may be, it must promiscuously interact with the various subtypes of K⁺ (and possibly other) channels expressed in glomus cells to redundantly modulate Ca²⁺ entry and transmitter release. Good candidates for broad O₂ modulation of membrane conductance are redox mediators, which could be produced in glomus cells upon exposure to decreased O₂ tension and thereby alter the redox state of several K⁺ channels, thus leading to cell depolarization. Although there is some experimental evidence against a role for redox in glomus cell O₂ sensing (for review and discussion see López-Barneo, 2003), redox-based O₂ sensing has been proposed for hypoxic pulmonary vasoconstriction (see Weir et al., 2005). Recently, hydrogen sulphide, a broadly distributed cytosolic gaseous reductant (Wang, 2002), has been suggested to participate in CB O₂ sensing through redox modulation of maxi-K⁺ channels in glomus cells (Li et al., 2010). Interestingly, internal application of reduced glutathione produces in single O₂-sensitive rabbit K⁺ channels biological alterations that resemble those induced by hypoxia (López-Barneo et al., 1998). As it occurs with O₂ sensing, CO₂ chemotransduction could depend on the change of internal pH and the redundant decrease of K⁺ conductance resulting from the protonation of residues in various subtypes of K⁺ channels.

Regardless of the nature of the mechanisms involved in O₂/CO₂ sensing, whose molecular characterization obviously needs further research, it is likely that what makes CB cells suitable for arterial chemoreception is their unique morphological and biophysical design. Glomus cells are only ~10 μm in diameter and have severalfold smaller surface areas and higher input resistances than other neural crest-derived cells, such as chromaffin cells or sympathetic neurons. Glomus cells contain several subtypes of background K⁺ channels that, combined with the expression of a standing Na⁺ current (Carpenter and Peers, 2001; García-Fernández et al., 2007), set their resting membrane potential to values (approximately −55 mV) near the activation threshold of voltage-dependent Na⁺ and Ca²⁺ channels. Hence, subtle changes in transmembrane current (that could be functionally negligible in other cell types with smaller resistances and more hyperpolarized resting potentials) might result in a depolarization of glomus cells of sufficient amplitude to activate Na⁺ and/or Ca²⁺ channels and trigger exocytosis.

We wish to express our gratitude to Dr. M. Isabel Aller and Prof. William Wisden (Imperial College, London, UK) for the gift of TASK1 and TASK3 knockout mice. Research was supported by the Spanish Ministry of Science and Health, the Marcelino Botín Foundation, and the Andalusian Government.

Lawrence G. Palmer served as editor.

Submitted: 20 July 2009
Accepted: 22 February 2010

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**Figure S1.** Fluorimetric recordings of intracellular Ca\(^{2+}\) concentration in dispersed mouse glomus cells using a confocal microscope. Cells were loaded with Fluo4-AM (5 μM for 30 min) and then bathed in the standard bicarbonate external solution equilibrated with air and exposed to either hypoxia (PO\(_2\) =15 mmHg) or 40 mM K\(^{+}\). For comparison, recordings from wild-type (A) and TASK1/3\/-/- (B) glomus cells are shown. Sample interval is 5 s. Calibration in ordinate in arbitrary units.

**Figure S2.** Effect of paxilline on potassium currents in dispersed neonatal rat chromaffin cell. (A) Representative traces of potassium current using the perforated patch configuration recorded before (black) and after (red) the addition of 500 nM paxilline. Holding potential, −80 mV. Depolarization voltages (mV) are indicated near each trace. Bath solution contained (in mM): 140 NaCl, 2.5 KCl, 2.5 CaCl\(_2\), 4 MgCl\(_2\), 10 HEPES, and 10 glucose; pipette solution: 70 K\(_2\)SO\(_4\), 30 KCl, 2MgCl\(_2\), 1 EGTA, and 10 HEPES with amphotericin B added. (B) Peak K\(^{+}\) current–voltage relation obtained from depolarization pulses from −80 to +100 mV, lasting 50 ms with 10-mV increments before (black) and after (red) the addition of 500 nM paxilline. Note that the characteristic “hump” in the current–voltage relation typical of the Ca\(^{2+}\)-dependent component of the current completely disappears after the addition of paxilline.