Respiratory Physiology & Neurobiology 177 (2011) 247–255

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Characterization of an ATP-sensitive K^+ channel in rat carotid body glomus cells

Donghee Kim[a,*], Insook Kim[b], Justin R. Papreck[a], David F. Donnelly[c], John L. Carroll[b]

[a] Department of Physiology and Biophysics, Rosalind Franklin University of Medicine and Science, The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064, United States
[b] Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, 1 Children's Way, Little Rock, AR 72202, United States
[c] Department of Pediatrics, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520, United States

ARTICLE INFO

Article history:
Accepted 17 April 2011

Keywords:
Ca^{2+}
Glomus cell
Carotid body
Chemoreceptor
K^+ channel

ABSTRACT

Carotid body glomus (CB) cells express different types of K^+ channels such as TASK, BK, and Kv channels, and hypoxia has been shown to inhibit these channels. Here we report the presence of a ~72-pS channel that has not been described previously in CB cells. In cell-attached patches with 150 mM K^+ in the pipette and bath solutions, TASK-like channels were present (~15 and ~36-pS). After formation of inside-out patches, a 72-pS channel became transiently active in ~18% of patches. The 72-pS channel was K^+-selective, inhibited by 2–4 mM ATP and 10–100 μM glybenclamide. The 72-pS channel was observed in CB cells isolated from newborn, 2–3 week and 10–12 week-old rats. Reverse transcriptase-PCR and immunocytochemistry showed that Kir6.1, Kir6.2, SUR1 and SUR2 were expressed in CB glomus cells as well as in non-glomus cells. Acute hypoxia (~15 mmHg O_2) inhibited TASK-like channels but failed to activate the 72-pS channel in cell-attached CB cells. K^+ channel openers (diazoxide, pinacidil, levcromakalim), sodium cyanide and removal of extracellular glucose also did not activate the 72-pS channel in the cell-attached state. The hypoxia-induced elevation of intracellular [Ca^{2+}] was unchanged by glybenclamide or diazoxide. NaCN-induced increase in [Ca^{2+}] was not affected by 10 μM glybenclamide but inhibited by 100 μM glybenclamide. Acute glucose deprivation did not elevate [Ca^{2+}] in the presence or absence of glybenclamide. These results show that an ATP-sensitive K^+ channel is expressed in the plasma membrane of CB cells, but is not activated by short-term metabolic inhibition. The functional relevance of the 72-pS channel remains to be determined.

1. Introduction

Carotid body glomus (CB) cells sense low pO_2 and initiate a cascade of events that ultimately leads to an increase in breathing and other cardiorespiratory responses. Initiation of excitation of CB cells by hypoxia is caused in large part by inhibition of background K^+ channels that allow cell depolarization to occur as a result of a resting a Na^+ conductance (Buckler, 2007; Carpenter and Peers, 2001). K^+ channels that give rise to the O_2-sensitive background K^+ conductance have generally been described to be TASK, BK and/or K_V channels (Buckler et al., 2000; Lopez-Barneo et al., 2004; Lopez-Lopez and Perez-Garcia, 2007; Peers and Wyatt, 2007). In isolated rat CB cells, TASK is the most active K^+ channel near the resting membrane potential, and is inhibited by hypoxia (Buckler et al., 2000; Kim et al., 2009). This is consistent with recent findings that the basal catecholamine secretion was increased in TASK-1/3 null mice and the carotid nerve response to hypoxia was reduced in these mice (Ortega-Saenz et al., 2010; Trapp et al., 2008). However, mice lacking TASK still show a hypoxia-induced increase in catecholamine secretion from the CB and increase in ventilation, indicating that other ion channels are involved in hypoxia sensing.

In many cell types including neurons, a decrease in [ATP] has been shown to activate an ATP-sensitive K^+ (K_{ATP}) channel. If present and active in the glomus cell, such activation would oppose the depolarization produced by leak channel inhibition, but these channels have not yet been identified in glomus cells. In the course of studying TASK function in excised patches of CB cells, we found the presence of an ion channel that was not open in cell-attached patches, but appeared after formation of inside-out and outside-out patches. In the present study, we characterized the biophysical properties of this channel. Single channel analysis and sensitivity of the channel by ATP and glybenclamide indicated that it is similar to those of ATP-sensitive K^+ channels previously described in cardiac and neuronal cells. However, K^+ channel openers failed to open the channel in CB cells. Acute hypoxia and glucose deprivation also did not cause activation of this K^+ channel. Therefore, it is unlikely that the 72-pS channel is involved in acute hypoxia-induced excitation

* Corresponding author. Tel.: +1 847 578 8356; fax: +1 847 578 3265.
E-mail address: donghee.kim@rosalindfranklin.edu (D. Kim).

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doi:10.1016/j.resp.2011.04.015

of CB. The potential role of this K^+ channel in chronic hypoxia and glucose deprivation remains to be determined.

2. Materials and methods

2.1. Carotid body cell isolation

The protocols for animal use in this study were approved by the Animal Care and Use Committees of Rosalind Franklin University and University of Arkansas for Medical Sciences. Rats (postnatal 14–18: 484 rats; 10–12 weeks: 15 rats) were anesthetized with isoflurane, decapitated, and the heads placed in ice-cold buffered saline solution (118 mM NaCl, 23 mM $NaHCO_3$, 3 mM KCl, 2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Glucose, pH 7.2). CBs from both sides were dissected and placed in ice-cold low-Ca^{2+}, low-Mg^{2+} phosphate buffered saline solution (low Ca^{2+}/Mg^{2+}-PBS: 137 mM NaCl, 2.8 mM KCl, 2 mM KH_2PO_4, 0.07 mM $CaCl_2$, 0.05 mM $MgCl_2$, pH 7.4). Each CB was cut into 3–4 pieces and placed in a solution containing trypsin (400 μg/ml) and collagenase (400 μg/ml) in low Ca^{2+}/Mg^{2+}-PBS and incubated at 37 °C for 20–25 min. CBs were gently triturated using a fire polished Pasteur pipette to mechanically dissociate the cells. Enzymatic digestion was continued for additional few minutes when necessary. CB growth medium (Ham's F-12, 10% fetal bovine serum, 23 mM glucose, 4 mM L-alanyl-glutamine (Glutamax-1), 10K units penicillin/streptomycin, and 300 μg/ml insulin) was added to stop enzyme activity. After brief trituration, the solution containing the digested CBs was centrifuged for 4 min at ~6000 rpm (~2000 × *g*) using a microcentrifuge. The supernatant was removed and warm CB growth media added to gently resuspend the pellet. This step was repeated to remove traces of enzymes. Suspended CB cells were placed on glass coverslips coated with polylysine, and incubated at 37 °C for 50 min to allow settling and attachment of the cells. CB growth medium was further added to the plate containing the coverslips and then incubated at 37 °C for additional 2 h. Coverslips were then transferred to the recording chamber for electrophysiological experiments.

2.2. Electrophysiological studies

Electrophysiological recording was performed using a patch clamp amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, CA). Channel current was filtered at 3 kHz using 8-pole Bessel filter (−3 dB; Frequency Devices, Haverhill, MA) and transferred to a computer using the Digidata 1320 interface at a sampling rate of 20 kHz. Single-channel currents were analyzed with the pCLAMP program (Version 10). Channel openings were analyzed to obtain channel activity (NP_o, where N is the number of channels in the patch, and P_o is the probability of a channel being open). NP_o was determined from ~20 s of current recording. The single channel current tracings shown in the figures were filtered at 1 kHz. In experiments using cell-attached and inside-out patches, pipette and bath solutions contained (mM): 150 KCl, 1 $MgCl_2$, 5 EGTA, 11 glucose and 10 HEPES (pH 7.3). In other cell-attached recordings for testing the effect of NaCN and K^+ channel openers, the bath solution contained (mM): 117 NaCl, 23 $NaHCO_3$, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 11 glucose (pH 7.3). The bath solution was quickly switched to that without $CaCl_2$ before forming inside-out patches to help detect the opening of the 72-pS channel. Outside-out patch was formed from the whole-cell configuration by gently lifting up the pipette. MgATP (0.1 mM) was added to the pipette solution when using outside-out patches. For statistics, Student's *t*-test (for comparison of two sets of data) was used with $p < 0.05$ as the criterion for significance. Data were analyzed using the Origin program and presented as mean ± S.D.

2.3. Intracellular [Ca^{2+}] measurement

Intracellular $[Ca^{2+}]_i$ was measured by quantitative epifluorescence imaging using the calcium-sensitive dye fura-2. Cells plated on the coverslip were incubated with 4 μM fura-2 acetoxymethyl ester (fura-2 AM; Molecular Probes) for 30 min at 37 °C. Fura-2 fluorescence emission was measured at 510 nm in response to alternating excitation at 340 and 380 nm. Images were acquired and stored using a NIKON TE300 microscope and CCD (CoolSNAP HQ_2) camera under computer control (MetaFluor: Molecular Devices). For each coverslip, the background light levels were determined and subtracted from each image before measurement of the fluorescence intensity ratio. $[Ca^{2+}]_i$ was determined using the 340/380 fluorescence ratio as described previously (Wasicko et al., 1999). Calibration was performed using cell-free solutions (Grynkiewicz et al., 1985).

2.4. Hypoxia studies

Cell-attached patches were formed on CB cells and perfused with a bicarbonate-buffered solution containing 117 mM KCl, 23 mM $NaHCO_3$, 1 mM $MgCl_2$, 11 mM glucose and 10 mM HEPES and gassed with 5% CO_2/95% air mixture (normoxia) for at least 60 min. After steady state channel activity was obtained, the perfusion solution was switched to solution gassed (for ~60 min) with 5% CO_2/95% N_2 mixture (hypoxia) for ~5 min (34 °C). Glucose oxidase (24 units/ml) and catalase (135 units/ml) were added to the hypoxic solution to further reduce O_2 pressure to <1% in one set of single channel recording experiments as indicated. The pipette solution contained (mM) 150 KCl, 1 $MgCl_2$, 5 EGTA, 10 glucose and 10 HEPES (pH 7.3). The temperature of the perfusion solutions was kept at ~34 °C, and the rate of perfusion was ~2.2 ml/min. Oxygen pressure of the solutions was checked using an oxygen meter (ISO2, WPI, Sarasota, USA) that was calibrated to 0% with solution gassed with pure nitrogen for 60 min and to 21% with solution gassed with air for 60 min at 34 °C. The O_2 partial pressure as judged by the reading on the meter for the hypoxic solution inside the recording chamber used in this study was ~2–3% (15–22 mmHg O_2).

2.5. Real time reverse-transcriptase (RT)-PCR (qPCR)

Total RNA was prepared from whole CB isolated from rats age P14–16. cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad). Using specific primers designed to yield a product of 100–150 bases, real time RT-PCR (qPCR) reaction was carried out for Kir6.1, Kir6.2B, SUR1, SUR2 and two reference genes, PPIA (peptidyl-proyl-isomerase A) and TBP (TATA-box binding protein). qPCR using SYBR green technology was performed on an iCycler iQ real-time detection system (Bio-Rad) in 96-well plates. The following amplification program was used: after 5 min of denaturation at 95 °C, 50 cycles of real time PCR with 2-step amplification were performed consisting of 15 s at 95 °C for denaturation, 45 s at 60 °C for annealing and 1 min at 95 °C for polymerase elongation. In each qPCR run, two reference genes and four K_{ATP} subtypes were run with cDNA in one 96 well PCR plate simultaneously. All samples were amplified in triplicate. The qPCR products after cleaning by MiniElute PCR purification kit (Qiagen) were run by 2% agarose gel electrophoresis to verify the expected qPCR products.

2.6. Immunocytochemistry

Dissociated CB cells of rats age P14–18 were plated on poly-D-lysine coated glass coverslip and cultured for 3–4 h in incubator. The cells were fixed with 4% paraformaldehyde and treated with PBS containing 0.4% Trion X-100 for 10 min at 24 °C. After wash with PBS, cells were incubated with rabbit polyclonal antibodies against