Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type 1 cells

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Williams, Beatrice A., and Keith J. Buckler. Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type 1 cells. Am J Physiol Lung Cell Mol Physiol 286: L221–L230, 2004. First published September 22, 2003; 10.1152/ajplung.00010.2003.—The single channel properties of TASK-like oxygen-sensitive potassium channels were studied in rat carotid body type 1 cells. We observed channels with rapid bursting kinetics, active at resting membrane potentials. These channels were highly potassium selective with a slope conductance of 14–16 pS, values similar to those reported for TASK-1. In the absence of extracellular divalent cations, however, single channel conductance increased to 28 pS in a manner similar to that reported for TASK-3. After patch excision, channel activity ran down rapidly. Channel activity in inside-out patches was markedly increased by 2 and 5 mM ATP and by 2 mM ADP but not by 100 μM ADP or 1 mM AMP. In cell-attached patches, both cyanide and 2,4-dinitrophenol strongly inhibited channel activity. We conclude that 1) whilst the properties of this channel are consistent with it being a TASK-like potassium channel they do not precisely conform to those of either TASK-1 or TASK-3, 2) channel activity is highly dependent on cytosolic factors including ATP, and 3) changes in energy metabolism may play a role in regulating the activity of these background K+ channels.

hypoxia; chemoreception; background K+ channels; ATP

Carotid body type 1 cells respond to both hypoxia and acidosis with membrane depolarization, voltage-dependent calcium entry, and neurosecretion (6, 7, 18, 32). Background K+ currents play a key role in mediating this membrane depolarization in response to both chemostimuli (4, 9). We have recently reported that this background K+ current shares strikingly similar pharmacology and biophysical properties to those of TASK-1, a cloned tandem-P domain K+ channel (9, 15). Similar endogenous TASK-like K+ currents have now been described in a number of other neuronal cell types, some of which may also have a chemosensory function (31, 37, 39). In many of these cells it is becoming increasingly apparent from studies using in situ hybridization and/or immunohistochemistry that they express more than one type of TASK channel, suggesting that endogenous TASK-like conductances may be heterogeneous. Similarly, recent immunohistochemical studies of the carotid body have indicated the presence of several other tandem-P domain K+ channels in type 1 cells in addition to TASK-1, including TASK-2, TASK-3, and TRAAK (42). Determining the extent to which different types of channel contribute to endogenous background K+ currents is difficult, if not impossible, by whole cell recording techniques, as many tandem-P domain K+ channels have similar pharmacological and biophysical properties. A recent study utilizing single channel recording has, however, successfully revealed that the TASK-like background K+ current in cerebellar granule cells comprises at least four distinct K+ channels, some of which show strong similarity with cloned TASK channels (19). We have therefore conducted a detailed study of the single channel properties of background K+ channels (Kb channels) in type 1 cells.

In addition to characterizing the Kb channels present in type 1 cells, we have also investigated mechanisms that might be responsible for modulating their activity. Over the years a number of different hypotheses have been advanced to explain oxygen sensing by the carotid body. Historically one of the most prominent is that oxygen sensing could be linked to mitochondrial metabolism (1). Inhibitors of electron transport and mitochondrial uncouplers are all potent chemostimulants (17) and inhibit K+ currents in these cells (8, 34). Moreover, the regulation of K+ channels by adenine nucleotide levels is a key feature of other metabolic sensors (2). This raises the possibility that cytosolic factors such as ATP, ADP, AMP, or Mg2+ may be involved in the regulation of Kb channels. Another hypothesis is that oxygen sensing could occur through the modulation of cell redox couples such as NADH/NAD+. Redox modulation of several voltage-sensitive K+ channels has also been reported (25, 36). We have therefore investigated the regulation of Kb channels by metabolism, adenine nucleotides, Mg2+, and NADH to gain some insights into possible mechanisms whereby Kb channel activity might be modulated.

METHODS

Cell isolation. Cells were enzymatically isolated from carotid bodies of neonatal rats (10–16 days old). The methods of cell isolation have been described in detail elsewhere (5). Briefly, rat pups were anesthetized with 4% halothane or isoflurane in oxygen, and the carotid bodies were quickly excised and placed in ice-cold saline. The rats were then killed by decapitation. Procedures involving animals were reviewed and approved by a University of Oxford Local Ethical Review Panel and by the United Kingdom Home Office. The carotid bodies were incubated in a phosphate-buffered saline containing collagenase (0.5 mg/ml, type 1; Worthington, Freehold, NJ) and trypsin (0.15–0.2 mg/ml, Sigma) at 37°C and then mechanically dispersed by the use of forceps followed by trituration through fire-polished glass pipettes. The cell suspension was then centrifuged, resuspended in Ham’s F-12 (supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 84 U/l insulin), and plated out onto coverslips coated with poly-d-lysine. Cells were maintained in culture medium until use (2–8 h). Type 1 cells were identified by their size and appearance under phase microscopy (see e.g., 14, 34).

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Electrophysiology. Experiments were performed using the cell-attached and inside-out configurations of the patch-clamp technique. Data were acquired at 10–20 kHz and filtered at 2–5 kHz, with an Axopatch 200B amplifier, Digidata 1200, and pClamp software (version 6.0 or 7.0, Axon Instruments). Electrodes were made from Clarke CG150 borosilicate glass capillaries (Clarke Electromedical, Pangbourne, Reading, UK) coated with Sylgard 184 (Dow Corning). Electrodes were fire polished before use and had resistances between 5 and 15 MΩ; seal resistances were >10 GΩ. In the cell-attached patch, the potential across the membrane (V_m) is the difference between the resting potential of the cell (V_c) and the potential applied to the inside of the pipette (V_p). The potential is expressed as negative, or inward, current (unless otherwise specified, i.e., Fig. 3Aa).

The bath was earthed via an Ag-AgCl pellet. Data are presented with correction for liquid junction and reference potential errors, which were measured to be between 0.3 and 10 nV for different combinations of pipette and bathing solutions.

Solutions. Cells were bathed in standard HCO_3^- buffered saline containing (mM): 117 NaCl, 4.5 KCl, 23 NaHCO_3, 1.0 MgCl_2, 2.5 CaCl_2, and 11 glucose bubbled with 5% CO_2 and 95% air; pH 7.4–7.45. For testing cyanide (CN^-) and 2,4-dinitrophenol (DNP) in cell-attached patches, we bathed cells in a modified calcium-free high-potassium HCO_3^- buffered saline containing (mM): 100 KCl, 17 NaCl, 23 NaHCO_3, 3.5 MgCl_2, and 11 glucose, which was bubbled with 5% CO_2 and 95% air. This solution was used to stabilize membrane potential and prevent large changes in intracellular calcium concentration ([Ca^{2+}]), since both DNP and CN^- depolarize type 1 cells and evoke large increases in [Ca^{2+}].

Extracellular pipette solutions for cell-attached and inside-out patches contained (mM): 140 KCl, 4 MgCl_2, 1 EGTA, 10 HEPES, and 10 tetraethylammonium (TEA)-Cl, pH 7.4 with KOH. The final pipette [K^+] was 146 mM. To test the ion selectivity of the channel, NaCl, N-methyl-D-glucamine (NMDG), or K_2SO_4 was substituted for KCl in the pipette.

Inside-out intracellular bath solutions contained (mM): 130 KCl, 5 MgCl_2, 10 EGTA, 10 HEPES, and 10 glucose. pH was adjusted to 7.2 with KOH. The final [K^+] was 152 mM.

The effect of high intracellular Ca^{2+} was tested on inside-out patches with an intracellular solution containing (mM): 130 KCl, 3.61 MgCl_2, 8.27 CaCl_2, 10 EGTA, 10 HEPES, and 10 glucose, pH 7.2 with KOH. This solution had a free [Ca^{2+}] of 1.0 mM, compared with ~0.03 mM in control. Free [Mg^{2+}] was maintained at 3.3 mM. The effect of reducing intracellular Mg^{2+} was tested on inside-out patches with an intracellular solution containing (mM): 130 KCl, 10 EGTA, 10 HEPES, and 10 glucose, pH 7.2 with KOH. (The free intracellular levels of Mg^{2+} and Ca^{2+} in this solution were estimated to be ~0.8 µM and 0.02 mM, respectively, based on the levels of Ca and Mg present as impurities in KCl.) Values for free intracellular divalent cation concentrations were calculated with published dissociation constants (29) and in-house software (P. Griffiths, University Laboratory of Physiology, Oxford).

TEA and 4-aminopyridine (4-AP) were added directly to the saline solutions, and the pH was adjusted as appropriate. Sodium cyanide and DNP were added directly to the saline solutions on the day of the experiment, and solutions were replaced every 1–2 h. K_2ATP, K_2ADP, and AMP were added to the intracellular solution directly on the day of the experiment, and the pH was adjusted as needed. In experiments with 2 and 5 mM K_2ATP, intracellular Mg^{2+} was increased to 7 and 10 mM, respectively, so that free intracellular [Ca^{2+}] was maintained at ~3 mM (MgATP was ~1.95 and 4.9 mM, respectively). After addition of nucleotides and adjustment of the pH of the intracellular solution, the [K^+] increased to a maximum of 174 mM with 5 mM ATP. Solutions were superfused at ~2 mL/min through a recording chamber with a volume of ~80 µL. Experiments were performed at 29–33°C.

Data analysis. Single channel recordings were analyzed with pClamp 6.0 software (Axon) or Spike3 (Cambridge Electronic Design). The value of channel amplitude was initially determined by eye using the amplitude of the longest open events. A Gaussian distribution was then fitted to level 1 open events to give an estimate of the single channel amplitude. Open events were defined using the 50% threshold criteria with events of 150%, 250%, etc. of threshold counted as multiple channel openings. Channel activity is reported as the open probability times the number of channels in a patch (NP_open). Kinetics and channel activity were also determined from the pClamp events list. For burst analysis, the minimum value of the closed duration that separates bursts of openings, the interburst interval, was determined using pClamp software, with bin width set to manual, test interburst interval of 0.1 ms, start value of 0.1 ms, and stop value of 2.0 ms. To verify the result, we also used the following equation (40), modified from Colquhoun and Sakmann (10):

\[
P_i(t > t_{\text{comp}}) = \int_{t_{\text{comp}}}^{\infty} \frac{A}{\tau_e} e^{-t/\tau_e} dt
\]

where \(P_i\) is the probability that a short closed time is greater than the interburst interval, \(t_{\text{comp}}\), and was set low, at 1%. \(A\) is the proportion of closed times that are assumed short and was obtained from the fit of the dwell time histograms. \(t_i\) is a closed time that is assumed short, and \(\tau_i\) is the mean measured short closed time (0.11 ms). The interburst interval was therefore taken as 0.6 ms.

Sigmplot 5.0 (SSPS, Chicago, IL) was used to calculate linear and nonlinear regressions of plotted data. Statistical significance of results was assessed by the Student’s t-test.

RESULTS

K_B channel activity in cell-attached patches. Single channel activity was routinely observed in cell-attached patches from type 1 cells at resting membrane potential (cells bathed in standard HCO_3^- buffered solution and pipette solution containing 146 mM K^+ and 10 mM TEA). The vast majority of recordings obtained under these conditions revealed at least one main open level of mean amplitude 1.16 ± 0.03 pA (n = 21) as well as other, less frequent, higher conductance levels (Fig. 1A). Few other forms of channel activity were observed in cell-attached patches at resting membrane potentials, save for the occasional appearance of a smaller channel with long-lasting openings. Due to the low frequency with which this second type of channel was observed, it was not studied further.

The current-voltage relationship of the main conductance state was linear, between ~120 and 30 mV pipette potential, and had a mean slope conductance of 15.6 ± 0.04 pS (n = 12) and a reversal potential of ~74 ± 2 mV (n = 10). Slight rectification was evident at pipette potentials more positive to 80 mV (approximately ~150 mV membrane potential) and at pipette potentials more negative to ~120 mV (50 mV membrane potential) (Fig. 1). These channels were active immediately and did not inactivate or run down over time.

Substitution of NaCl for KCl, in the pipette, led to a rightward (positive) shift in the single channel current-voltage relationship and the appearance of marked rectification at low extracellular K^+ concentration ([K_L]) (Fig. 1B). Reversal potentials were estimated by linear interpolation of 3–8 points nearest the x-axis. A plot of reversal pipette potential vs. log
[K] e had a linear slope of $-55 \pm 2$ mV/decade (Fig. 1C). (On converting pipette potential to membrane potential the slope value changes sign to $55 \pm 2$ mV/decade.) This is very close to that expected for a purely K$^+$-selective channel (slope of 60 mV/decade at 30°C).

To confirm that the channels were indeed K$^+$ selective, we also performed experiments in which the reversal potential was determined at different concentrations of extracellular (pipette) chloride and sodium. Reduction of extracellular chloride from 158 to 30 mM (by substitution of KCl with extracellular K$_2$SO$_4$) had no significant effect on the reversal potential or on the slope conductance measured between $-120$ and 40 mV pipette potential ($-74 \pm 2$ mV and $16 \pm 1$ pS in 158 mM Cl$^-$, $n = 10$; compared with $-68 \pm 3$ mV and $15 \pm 1$ pS in 30 mM Cl$^-$, $n = 6$). Indeed, the single channel current-voltage relationships obtained in low and normal chloride were superimposable (Fig. 2A). Similarly, replacing 70 mM Na$^+$ with 70 mM NMDG (pipette K$^+$ = 70 mM) had no significant effect on the reversal potential or the current-voltage relationship (reversal potential = $-49 \pm 8$ mV with NMDG, $n = 4$; compared with $-56 \pm 2$ mV with Na$^+$, $n = 10$). Thus substantive changes in pipette chloride or sodium failed to have
any affect on single channel current-voltage relationships, confirming that these channels were primarily K⁺ selective.

**Inside-out patches.** Continuous recordings of channel activity from patches in the cell-attached configuration through patch excision to the inside-out configuration revealed a rapid rundown in channel activity (Fig. 3A). For these studies cells were superfused with the standard inside-out recording medium (see METHODS) after seal formation and for at least 10–15 s before patch excision to obtain continuous recordings of channel activity before, during, and after patch excision. Within the limits of resolution of the timing of patch excision (approximately ± 1-s), rundown appeared to begin immediately and was complete within 40 s of patch excision with channel activity having fallen to ~10% of the cell-attached level (Fig. 3B). Despite this rundown single channel, events were clearly and routinely observed in inside-out patches with 146 mM [K⁺]ᵢ in the pipette solution and 152 mM [K⁺]ₑ (Fig. 3B) and had a single channel amplitude at ~76 mV of 1.15 ± 0.06 pA (n = 7).

The current-voltage relationship of these channels was determined in the inside-out patch with 146, 100, and 70 mM [K⁺]ₑ in the pipette. With 146 mM [K⁺]ₑ and 152 mM [K⁺]ᵢ, the current-voltage relationship was linear, in the range 40 to ~100 mV, with a slope conductance of 14 ± 0.9 pS (n = 6) and a reversal potential of 8 ± 2 mV. Weak rectification was again evident in inside-out patches, as with cell-attached patches. This rectification did not decrease over time, suggesting it is not due to the presence of diffusible polyamines (26).

As in cell-attached patches, the reversal potential was shifted toward more negative potentials as [K⁺]ᵢ was decreased consistent with a K⁺-selective channel (Fig. 3, C and D). Reversal potentials were measured by linear interpolation and a plot of reversal potential against log [K⁺]ₑ could be fitted with a linear slope of 60 ± 5 mV (Fig. 3C).

We again performed a number of recordings in which extracellular chloride and sodium were reduced to confirm the K⁺ selectivity of the channels observed in excised patches. As in cell-attached patches, reduction of extracellular chloride from 158 to 30 mM had no significant effect on reversal potential (8 ± 2 mV in 158 mM Cl⁻, n = 6; compared with 4 ± 2 mV in 30 mM Cl⁻, n = 6). Similarly, reduction in extracellular Na⁺ from 70 mM to 0 (NMDG substituted) had no effect on single channel conductance or reversal potential (~17 ± 3 mV in 0 Na⁺, n = 5; compared with ~10 ± 3 mV in 70 mM Na⁺, n = 5; Fig. 2B).

Thus aside from a lower level of activity, the single channels observed in excised inside-out patches appeared to be similar to those observed in cell-attached patches. The strong biophysical similarities between the channel activity seen in the cell-attached patch and that seen in the inside-out patch suggest that the channels observed under these different recording conditions are one and the same. In view of the high K⁺ selectivity of these channels and the fact that they are open at all potentials, they will be termed Kᵦ channels.

**Effects of TEA and 4-AP.** TEA at 10 mM was present in the pipette solution in all above single channel recordings. Removal of TEA from the extracellular solution had no effect on single channel conductance or channel activity (NP_open = 0.30 ± 0.1, n = 7 -TEA; 0.27 ± 0.04, n = 29 +TEA; measured at 0 mV), nor did it visibly alter the channel kinetics.

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**Fig. 3.** Single channel activity from inside-out patches. A: a: representative single channel recording showing rundown of activity following patch excision of a cell-attached patch to form an inside-out patch. Patch excision occurred at 10 ± 1 s; Vᵢ was maintained at 70 mV throughout. Note that in this trace inward currents through channel openings are represented as upward deflections from baseline. A, b: average time course of rundown following patch excision. Mean ± SE NP_open determined over 2-s intervals relative to NP_open determined over entire cell-attached period for 11 patches. The bath solution contained (mM) 140 KCl, 10 HEPES, 5 mM MgCl₂, 10 EGTA, and 10 glucose and was maintained at pH 7.2 at 33°C. Pipette potential was maintained at 70 mV throughout. B: single channel recording from an inside-out patch at ~70 mV with 146 mM extracellular and 152 mM intracellular [K⁺], in the absence of intracellular ATP. C: inside-out single channel I-V relationship. Patches were bathed in 152 mM [K⁺]ᵢ solution, with no ATP, and the pipette contained 146 mM [K⁺]ₑ (n = 7), 100 mM [K⁺]ₑ (n = 5), or 70 mM [K⁺]ₑ (n = 5). The dashed lines are the best fit of the GHK current equation to the mean data. The K⁺ permeability coefficient was calculated to be 2.8 ± 0.2 × 10⁻¹⁴ cm⁻²s⁻¹. D: reversal potential plotted against [K⁺]ᵢ (logarithmic scale) for inside-out patches. Reversal potentials were determined by linear interpolation of the 3–8 points nearest the x-axis. Numbers in parentheses are the number of experiments used in each determination.
inward and outward currents, respectively; channel activity (107 of 10 mM extracellular TEA), however, had no effect on the intracellular surface of inside-out patches (in the presence also being insensitive to extracellular TEA, is strongly inhibitory to background K+ channels were insensitive to extracellular TEA. In cerebellar granular, pipette, 4-AP had no signiﬁcant effect on single channel openings (often of variable size), and there was more apparent channel activity and rectiﬁcation than for inward currents. By contrast, in the presence of extracellular 4-AP (in addition to TEA), outward currents were more clearly resolved (Fig. 4C), and inward rectiﬁcation was signiﬁcantly reduced (P < 0.05, n = 5, Fig. 4B). Moreover, under these conditions channel activity was independent of voltage from −140 to 40 mV (9). Thus the apparent voltage sensitivity of channel activity and rectiﬁcation seen in the absence of extracellular 4-AP is probably due to the presence of other, contaminating, voltage-gated 4-AP-sensitive K+ channels in the same patch.

Kinetics of the K_B channel. It is clear from visual inspection that the K_B channel has very rapid single channel kinetics, consisting of short bursts of rapid openings (Fig. 5). Kinetic parameters were measured from cell-attached recordings acquired at 20 kHz and ﬁltered at 5 kHz. In these, as well as all other patch clamp recordings, both whole cell and inside out, we failed to obtain any recordings in which there were no double, or higher, conductance state openings (see DISCUSSION). Consequently measurements were taken from those recordings in which large sections of trace were of one main single channel level, and multiple openings (or higher conductance states) were rare.

of the inward currents (9) (Fig. 4A), conﬁrming that, like the whole cell background K+ current (4), these background channels were insensitive to extracellular TEA. In cerebellar granule neurons, one of the channels thought to be a major contributor to background K+ currents, the type 4 channel, whilst also being insensitive to extracellular TEA, is strongly inhibited by intracellular TEA (19). Application of 20 mM TEA to the intracellular surface of inside-out patches (in the presence of 10 mM extracellular TEA), however, had no effect on channel activity (107 ± 21% and 96 ± 5% of control for inward and outward currents, respectively; n = 3) or single channel amplitude (91 ± 7% and 104 ± 7% of control for inward and outward currents, respectively; n = 3). The K_B channels of type 1 cells are therefore clearly different from the type 4 channel of cerebellar granule neurons.

The effect of 5 mM 4-AP was also tested with 10 mM extracellular TEA present. In cell-attached patches, extracellular, pipette, 4-AP had no signiﬁcant effect on single channel conductance at negative membrane potentials (16.7 ± 0.6 pS with 4-AP, n = 5; 16 ± 1 pS in control, n = 10, Fig. 4B), reversal potential (−61 ± 6 mV pipette potential, n = 5), or channel activity (measured at 0 mV pipette potential, i.e., resting membrane potential, N/PoPen = 0.21 ± 0.06 , n = 9 with 4-AP; 0.27 ± 0.04 , n = 29 control). In the absence of extracellular 4-AP, however, outward channel openings were less well deﬁned than inward channel openings (often of variable size), and there was more apparent channel activity and baseline noise than for inward currents. By contrast, in the presence of extracellular 4-AP (in addition to TEA), outward currents were more clearly resolved (Fig. 4C), and inward rectiﬁcation was signiﬁcantly reduced (P < 0.05, n = 5, Fig. 4B). Moreover, under these conditions channel activity was independent of voltage from −140 to 40 mV (9). Thus the apparent voltage sensitivity of channel activity and rectiﬁcation seen in the absence of extracellular 4-AP is probably due to the presence of other, contaminating, voltage-gated 4-AP-sensitive K+ channels in the same patch.

**Fig. 4.** Effects of extracellular TEA and 4-aminopyridine (4-AP) on the K_B channel. A: single channel recordings from 3 different cell-attached patches at resting membrane potential, showing that addition of 10 mM TEA and 10 mM TEA + 5 mM 4-AP had no effect on inward channel activity. B: I-V plot for cell-attached patches. In the presence of 10 mM TEA + 5 mM 4-AP, inward rectiﬁcation was signiﬁcantly reduced compared with that in the presence of 10 mM TEA alone (c, n = 21). C: single channel recording from a cell-attached patch recorded with 146 mM K+ pipette solution containing 5 mM 4-AP and 10 mM TEA. Potentials shown are pipette potentials, and the arrows point to the zero current level. Traces are typical of 5 separate experiments.

**Fig. 5.** The K_B channel has ﬂickery kinetics. A: representative single channel recording from a cell-attached patch held at V_P = 70 mV. The dotted line follows the closed state. B. a: open-time histogram from the cell-attached patch shown in A. The histogram was best ﬁtted with a single exponential (solid curve) with a time constant, τ_o = 0.19 ms. b: representative closed-time histogram from the same patch as in A. The short closed-time constant, τ_c = 0.14 ms, and the long time constant, τ_L = 20 ms. Note the x- and y-axes have log and square root scales, respectively.
Open time histograms were best fitted with a single exponential and were corrected for sampling and bin promotional errors with pClamp6 software (Fig. 5Ba). At 70 mV pipette potential in cell-attached patches, the mean open time constant, \( \tau_o \), was 0.28 \pm 0.02 ms \((n = 15)\). Mean open times were independent of pipette potential between 0 and 100 mV \((\tau_o = 0.27 \pm 0.04 \text{ ms} \ (n = 5) \) at 0 mV and 0.29 \pm 0.02 ms \((n = 10) \) at 100 mV).

Closed time histograms were best fitted with a double exponential, consistent with the channel displaying bursting kinetics (Fig. 5Bb). Due to relatively short lengths of recording and the possible presence of multiple channel levels, the measured duration of the long closed state was very variable and could be anywhere between 2 and 30 ms. The variability in long closed time may, in part, be a consequence of varying numbers of channels in the patch. It is also possible, however, that modulation of channel activity could involve changes in the stability of the long closed state. The mean short closed time was 0.11 \pm 0.08 ms at 70 mV pipette potential (binned logarithmically without correction for sampling promotional errors). Values for both the mean open time and the mean short closed time are longer than the true values since the time constants are close in value to the filter dead time (0.04 ms) (11). Burst durations were measured to be \( \sim 1.7 \pm 0.3 \text{ ms} \ (n = 12) \) at 70 mV pipette potential. Burst durations were also independent of pipette potential between 0 and 100 mV \((1.8 \pm 0.4 \text{ ms} \ (n = 4) \) at 0 mV, and 1.3 \pm 0.1 ms \((n = 8) \) at 100 mV). The burst duration should be fairly close to the true value since the errors in open time are primarily due to missed gaps within a burst (11).

We have previously reported that \( K_B \) channel activity is reversibly inhibited by hypoxia in cell-attached patches (9, 41). We have therefore also analyzed the effect of hypoxia on channel kinetics. In cell-attached patches, hypoxia significantly increased the long closed time \((166 \pm 26\% \text{ of control, } P < 0.05, n = 9) \) without affecting mean open time \((103 \pm 6\%) \), mean short closed time \((85 \pm 11\%) \), or mean burst duration \((99 \pm 7\%) \).

Regulation of \( K_B \) channels: effects of divalent cations on the \( K_B \) channel. Because \([Ca^{2+}]\) was buffered to very low levels in our standard intracellular media \((10 \text{ mM EGTA, no added Ca}^{2+})\), below that normally found at rest in these cells \((100 \text{ nM, Ref. 5)}\), it was important to establish whether the \( K_B \) channel was sensitive to changes in \([Ca^{2+}]\), and whether the fall in \([Ca^{2+}]\), upon patch excision might account for channel rundown. In inside-out patches, increasing intracellular \( Ca^{2+} \) from 0.03 nM to 1 \text{ \mu M} \) had no significant effect on channel activity or single channel amplitude (Fig. 6, A and B); single channel amplitude at \(-100 \text{ mV} \) was 1.30 \pm 0.07 pA in control and 1.35 \pm 0.08 pA with 1 \text{ \mu M} \( Ca^{2+} \). Outward current channel activity in the presence of 1 \text{ \mu M} \( Ca^{2+} \) was 86 \pm 15\% of that in control \((n = 4)\). These results confirm that the \( K_B \) channel is not directly \( Ca^{2+} \)-activated. Thus the rundown in channel activity upon patch excision is unlikely to be due simply to the reduction in \([Ca^{2+}]\).

The effect of reducing intracellular \( Mg^{2+} \) on channel activity was also tested. Neither the single channel current-voltage relationship nor channel activity was affected by a reduction in \([Mg^{2+}]\), from 3.3 mM to 0.8 \text{ \mu M} \ (Fig. 6D; in low \([Mg^{2+}]\),

Fig. 6. The effect of divalent cations on the \( K_B \) channel. A: single channel recording from an inside-out patch at \(-100 \text{ mV} \) showing that increasing \([Ca^{2+}]\), from nominally zero \((-0.03 \text{ nM}) \) to 1 \text{ \mu M} \) had no effect on channel activity. B: summary of the effect of increasing \([Ca^{2+}]\), to 1 \text{ \mu M} \) on inside-out patches \((n = 10)\). C: reducing extracellular \( Mg^{2+} \) from 3.7 mM to nominally free \((-0.9 \text{ \mu M}) \), while maintaining the same \([Ca^{2+}]\), increased single channel currents. Two separate single channel recordings \((\pm Mg^{2+})\) from patches held at \(+60 \text{ mV} \ V_m \) (approximately \(-120 \text{ mV} \ V_m \)) in the cell-attached configuration. D: effects of reducing extracellular \( Mg^{2+} \) on single channel \( I-V \) relationship \((\text{a, b, c})\); slope conductance 28 \pm 3 \text{ \mu S}, \( n = 4 \); data from cell-attached patches had potentials corrected to approximate patch \( V_m \) to facilitate comparison with recordings from inside-out patches and reducing free intracellular \( Mg^{2+} \) from 3 mM to 0.8 \text{ \mu M} \ (inside-out patch, \( n = 21 \)). Note that whilst reducing extracellular \( Mg^{2+} \) increased the conductance for inward current, reducing intracellular magnesium had no effect on either inward or outward currents; i.e., the weak inward rectification seen under control conditions was not reduced by a reduction in intracellular \( Mg^{2+} \).
channel activity was 104 ± 10% and 97 ± 5% of control for inward and outward currents, respectively (n = 5). In contrast, reducing extracellular Mg$^{2+}$ from 3.7 mM to ~0.9 μM (nominal Mg$^{2+}$ free) significantly increased single channel conductance to inward currents from 15.6 ± 0.4 pS (n = 12) to 28 ± 3 pS (n = 4; P < 0.05; Fig. 6C).

**Regulation of K$_B$ channels: effect of intracellular nucleotides.** Rundown in ion channel activity following patch excision has been observed for many different ion channels and is often associated with the loss of cytosolic ATP. We therefore tested the effects of 2 and 5 mM ATP on inside-out patches at least 1 min following patch excision (i.e., after rundown). In the majority of patches studied, ATP induced a clear, and reversible, increase in single channel activity of approximately fourfold in the presence of 5 mM ATP (Fig. 7, A and C); NP$_{open} = 0.04 ± 0.01$ control and 0.14 ± 0.03 ATP (n = 18, P < 0.005). At 5 mM, ATP had no significant effect on mean open times, albeit measured from data obtained with a 2-kHz bandwidth, but significantly decreased the long closed time to 17 ± 3% of control (n = 7, P < 0.05). We also investigated the effects of ADP and AMP on channel activity. At a high physiological concentration of 100 μM, ADP had no discernable effect on single channel activity in the excised patch; NP$_{open} = 0.05 ± 0.02$ control and 0.07 ± 0.03 plus AMP (n = 5, Fig. 7C). At much higher concentrations, however, ADP mimicked the effects of ATP; i.e., 2 mM ADP reversibly increased channel activity in the majority of patches by, on average, twofold (Fig. 7, B and C); NP$_{open} = 0.05 ± 0.01$ control and 0.10 ± 0.03 in the presence of ADP (n = 13, P < 0.01, see Fig. 7). Application of 1 mM intracellular AMP had no significant effect on channel activity (Fig. 7C); mean NP$_{open} = 0.03 ± 0.004$ control and 0.02 ± 0.004 plus AMP (n = 6).

The possibility that the channel might also be regulated by a change in the redox state of the cell was tested on inside-out patches by application of 2 mM intracellular NADH in the presence of 2 mM ATP. There was no significant effect of NADH on channel activity (control = 0.09 ± 0.05, NADH = 0.08 ± 0.04, wash = 0.05 ± 0.02; n = 3).

**Regulation of K$_B$ channels: metabolic regulation of the channel.** Given the sensitivity of K$_B$ channel activity to ATP in excised patches, an obvious question is whether changes in metabolism could play a role in the regulation of channel activity in vivo. We therefore examined the effects of two classical inhibitors of energy metabolism on K$_B$ channel activity in cell-attached patches. For these experiments cells were bathed in a calcium-free high-K$^+$ Tyrode solution to depolarize and stabilize membrane potential and prevent large changes in [Ca$^{2+}$$]$.

Application of 2 mM ClCN$^-$, an electron transport inhibitor, rapidly and reversibly inhibited channel activity in cell-attached patches by 56 ± 10% (n = 8, P < 0.05; Fig. 8). The mitochondrial uncoupler DNP (250 μM) also caused a rapid and reversible inhibition of channel activity in cell-attached patches by 57 ± 10% from that in control (n = 8, P < 0.05; Fig. 8). DNP had no effect on mean open time or mean short closed time but significantly increased the mean long closed time by 370 ± 68% (kinetics determined from data obtained with a 2-kHz filter). In contrast, when applied to the intracellular side of inside-out patches, neither DNP nor CN$^-$ had any effect on channel activity (Fig. 8).

**DISCUSSION**

**Resting membrane potential of the carotid body.** Only relatively recently have chemoreceptor cells been considered excitable cells, with membrane potentials negative to approximately ~50 mV and dependent on potassium (4, 14, 17, 20). Our measurements of single channel activity allow us to make another estimate of resting membrane potential. The Goldman-Hodgkin-Katz (GHK) equation was fitted to data obtained in the inside-out patch by a curve-fitting routine (Sigma Plot, Marquardt-Levenberg algorithm). A good fit ($r^2 = 0.84$) was obtained to data, between −120 and 50 mV, at 146, 100, and 70 mM [K$^+$], (Fig. 3). The K$^+$ permeability coefficient thus calculated was $2.8 ± 0.2 × 10^{-14}$ cm$^3$/s. This value for the
performed. There was no significant whole cell background, or leak, K
channel permeability coefficient was then used to fit the GHK
equation to the data obtained in the cell-attached configuration,
between −120 and 30 mV pipette potential, at 146, 100, 70,
and 30 mM pipette K+. The results of this procedure gave a
good fit ($r^2 = 0.97$) to the cell-attached data (Fig. 1) and
yielded estimates for membrane potential and [K+]1, of −81 ±
2 mV and 163 ± 13 mM, respectively, supporting the conclusion
that these cells normally maintain a relatively negative
resting membrane potential.

Comparison of background channels with the oxygen-sensitive $K_B$ current. The properties of the $K_B$ channel described
here correspond closely with those of the oxygen-sensitive
whole cell background, or leak, $K^+$ current recorded by Buc-
kler (4) in neonatal rat carotid body type 1 cells. Both the $K_B$
channel and the whole cell $K^+$ current are insensitive to 10 mM
TEA and 5 mM 4-AP (at negative potentials) and show an
almost linear current-voltage relationship in symmetrical K+
concentrations in the presence of these drugs (9). The $K_B$
channel and the whole cell $K^+$ current are both inhibited by
hypoxia to a similar extent (a reduction of $35 \pm 5\%$ compared
with $40 \pm 6\%$ in the whole cell; see Ref. 9), and both currents
are inhibited by 250 μM DNP, a mitochondrial uncoupler, to
a similar extent (a reduction of $57\%$ for the $K_B$ channel vs. $56\%$
for the whole cell $K^+$ current; Ref. 8). Finally, both the $K_B$
channel and the whole cell $K^+$ current is activated by the
general anesthetic halothane and inhibited by the local anes-
thetic bupivacaine (9). These observations support the conclu-
sion that the channels described here are primarily responsible
for the oxygen-sensitive background $K^+$ conductance of car-
rotid body type 1 cells (4).

Fig. 8. 2,4-Dinitrophenol (DNP) and cyanide (CN) inhibit channel activity in
the cell-attached patch but not in the inside-out patch. A: single channel
cell-attached recordings showing inhibition of activity by 250 μM DNP, a
mitochondrial uncoupler (top trace), and 2 mM CN (bottom trace). DNP and
CN were applied in separate experiments. Cells were bathed in calcium-free
high-K+ Tyrode solution and held at a $V_m$ of 70 mV. B: summary of effects of
metabolic inhibitors on the $K_B$ channel. The effect of hypoxia is shown for
comparison. Filled bars are from cell-attached patches, and open bars are from
inside-out patches. *Significant decrease in channel activity compared with
control ($P < 0.05$); the numbers in parentheses are the number of experiments
performed. There was no significant effect in inside-out patches, consistent
with an intracellular factor being involved in regulating channel activity in the
intact cell.

Single channel properties/kinetics. The $K_B$ channel in type 1
cells has a unitary conductance for the main open state of $\sim 15$
pS as measured for inward currents over a range of negative
membrane potentials in a symmetrical K+ gradient. Whilst this
was the predominant form of channel activity seen, visual
inspection of all patches showing such channels (>300) also
revealed the presence of brief openings to higher conductance
levels in addition to the 15-pS level. In none of our recordings,
however, did we see similar higher conductance channel open-
ings in isolation (i.e., without the lower 15-pS openings). The
higher conductance levels observed could therefore represent
the opening of more than one channel simultaneously or
different conductance states of the same channel. Although
some patches inevitably contained multiple channels, variable
conductance levels were also present in patches with very low
overall channel activity. This observation suggests that higher
conductance levels are unlikely to be solely due to coincident
openings of more than one channel and may therefore represent
multiple conductance states for the same channel. Thus in
contrast to the study of Han et al. (19) on cerebellar neurons,
we were able to identify only one major channel type that could
be associated with the TASK-like background $K^+$ current in
type 1 cells.

Studies on channel gating revealed that the $K_B$ channel
opened in short bursts with a mean duration of $\sim 2$ ms com-
prising a single brief open state (0.5 ms for the main
conductance level) and even briefer closings (∼0.1 ms). These
bursts were interspersed with a highly variable long closed
time. These measurements suggest a minimal kinetic model of
one open state and two closed states. Channel modulation by
hypoxia appeared to be mediated primarily through changes in
the stability/duration of the long closed state with no detectable
effect on conductance, $\tau_{cw}$, short closed time constant, or burst
duration.

Identity of the $K_B$ channels. As previously described (9),
there is compelling evidence to suggest that this oxygen-
sensitive background $K^+$ current/channel is a TASK-like
member of the two-P domain $K^+$ channel family. Here we
have further shown that unitary outward currents at positive
potentials (>50 mV) were unaffected by reducing [Mg2+],
supporting the notion that this channel is not a weakly recti-
fying inwardly rectifying $K^+$ channel (30) and that increasing
[Ca2+]1 had no effect on channel activity, confirming that it is
not a Ca2+-activated $K^+$ channel.

The biophysical properties of the $K_B$ channel show a number
of similarities with those of the cloned channel TASK-1: i.e.,
1) TASK-1 has a main single channel conductance of 13–16 pS
for inward currents and what may be larger subconductance
states (23, 27), 2) weak inward rectification of single channel
conductance at positive potentials that is insensitive to intra-

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cellular magnesium, 3) brief open times of 0.7–0.3 ms, and 4) at least two closed states (23, 27). There are, however, also some differences between the properties of the $K_B$ channels observed in type 1 cells and those of TASK-1. One possibly minor point of difference is the much briefer short closed times of the $K_B$ channel (0.11 ms) compared with that reported for TASK-1 (5 ms, Ref. 27). These kinetic parameters may, however, be subject to both intracellular modulation and methodological error in their determination due to limiting bandwidth of single channel recordings. A more striking observation, however, was that removal of extracellular magnesium (in 0 mM $Ca^{2+}$) doubled unitary conductance for inward currents (see Fig. 6D). Similar observations have also been reported for TASK-3, i.e., that removal of extracellular divalent cations increases the conductance to inward current (35), whereas whole cell TASK-1 currents appear to be unaffected by the removal of extracellular divalent cations (27, 28, J. Cochran and K. J. Buckler unpublished observations). Although TASK-1 and TASK-3 share a number of common features, which makes any distinction between them difficult, one key difference is in the single channel conductance. TASK-3 is reported to have a single channel conductance for inward currents of 27 pS in 2 mM external $Mg^{2+}$ (0 mM $Ca^{2+}$) or 1 mM $Mg^{2+}$ and 1 mM $Ca^{2+}$ (22, 35), 36 pS in 1 mM $Mg^{2+}$ (19), and 100 pS in 0 $Mg^{2+}$ (35); these values are clearly much higher than those obtained for the $K_B$ channel (15 pS in 4 mM $Mg^{2+}$ and 28 pS in 0 $Mg^{2+}$ and 0 $Ca^{2+}$). It would therefore seem unlikely that the $K_B$ channel is a simple TASK-3 homomer.

The abrupt rundown of activity of the $K_B$ channel following patch excision. In this respect the $K_B$ channels studied here differ from both cloned TASK channels and endogenous TASK-like channels in cerebellar granule neurons that have been reported to be insensitive to cytosolic ATP (19, 23). Some endogenous TREK-like channels, however, have also been found to be activated by intracellular ATP, unlike heterologously expressed TREK-1 (16, 38). ATP sensitivity would therefore appear to be conferred on some tandem-$p$ domain channels when expressed endogenously. This raises the question as to whether this ATP sensitivity results from interaction with a regulatory subunit.

We also noted that both CN$^-$ and the mitochondrial uncoupler DNP markedly reduced background channel activity in cell-attached patches (but not in excised patches). This observation suggests that changes in cytosolic ATP levels could play a role in modulating channel activity in the intact cell. During metabolic inhibition the rise in ADP is expected to be relatively small compared with fall in ATP; for example, during CN$^-$ poisoning of the heart ATP falls from 8 to 4.8 mM but ADP rises only from 40 to 100 $\mu$M (24).

The above observations inevitably lead to the question as to whether oxygen sensing itself could be mediated via changes in oxidative phosphorylation and cellular ATP levels. Of central importance to this issue is the question as to whether the oxygen sensitivity of mitochondrial metabolism is sufficient to account for the oxygen-dependent regulation of channel activity, and there is some evidence to suggest that this may be the case (13). At this stage, however, there is no direct proof that oxygen sensing by $K_B$ channels does indeed involve inhibition of mitochondrial ATP synthesis. Our observations do, however, permit a number of predictions to be made that should allow this hypothesis to be tested. For example, if this hypothesis were true, it should be demonstrable 1) that as hypoxia and mitochondrial inhibitors modulate the same channels then their effects on channel activity should be mutually exclusive; 2) that levels of hypoxia that inhibit channel function should also impair mitochondrial function sufficiently to lower cytosolic ATP levels; and 3) that appropriate, direct manipulation of adenine nucleotide levels should prevent changes in channel activity during hypoxia. These issues now need to be addressed.

Conclusions. In summary, this work gives further support to the idea that the oxygen-sensitive $K^+$ channel in neonatal rat carotid body type 1 cells is a TASK-like background $K^+$ channel. The biophysical properties of these channels do not, however, precisely conform to those of either TASK-1 or TASK-3, suggesting that the $K_B$ channels are not simple homomultimers of TASK-1 or TASK-3.

The activity of these $K_B$ channels is highly dependent on cytosolic factors including ATP and is also sensitive to the inhibition of oxidative phosphorylation. Changes in cellular energy metabolism could therefore play a key role in regulating the activity of these channels. The extent to which changes in metabolism might be involved in the oxygen-dependent regulation of $K_B$ channel activity, however, remains to be determined.

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