Regulation of recombinant human brain tandem P domain K⁺ channels by hypoxia: a role for O₂ in the control of neuronal excitability?

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Abstract

The tandem P domain potassium channels, TREK1 and TASK1, are expressed throughout the brain but expression patterns do not significantly overlap. Since normal pO₂ in central nervous tissue is as low as 20 mmHg and can decrease even further in ischemic disease, it is important that the behaviour of human brain ion channels is studied under conditions of acute and chronic hypoxia. This is especially true for brain-expressed tandem P-domain channels principally because they are important contributors to neuronal resting membrane potential and excitability. Here, we discuss some recent data derived from two recombinant tandem P-domain potassium channels, hTREK1 and hTASK1. Hypoxia represents a potent inhibitory influence on both channel types and occludes the activation by arachidonic acid, intracellular acidosis and membrane deformation of TREK1. This casts doubt on the idea that TREK1 activation during brain ischemia might facilitate neuroprotection via hyperpolarising neurons in which it is expressed. Interestingly, hypoxia is unable to regulate alkalotic inhibition of TREK1 suggesting that this channel may be more intimately involved in control of excitability during physiological or pathological alkalosis.

Keywords: potassium channels • ion channels • brain ischemia

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Oxygen sensing by tandem P domain potassium channels

One of the most recent families of ion channel genes to have been identified encode 15 or so members of the tandem P domain potassium channels [1]. The products of these genes are widely expressed but demonstrate considerable tissue selectivity [1, 2]. Although the physiological functions beginning to be ascribed to members of this potassium channel gene family are incredibly diverse, most are a direct result of their ability to control tightly the resting membrane potential of cells in which they are expressed. For example, TREK1 (KCNK2) has been proposed to control hippocampal neuronal excitability and, via its polymodal modulation by acidosis and long chain fatty acids, to be crucial to neuronal survival during ischemic insult. TASK1 (KCNK3) is expressed in cerebellum where it underlies the standing outward current and, as a consequence, is intimately involved in controlling granule cell excitability [3, 4]. Tandem P domain potassium channel expression is not confined to the central nervous system and evidence is beginning to emerge which implicates TASK1 and TASK3 in the chemoreceptor response to hypoxia [5, 6]. Thus, the background conductance of both the carotid body glomus cell [5] and an immortalized airway receptor cell line is acutely suppressed by hypoxia [6, 7] which generates a depolarizing stimulus of sufficient magnitude to elicit calcium influx and amine release. Recently, the combined strategy of loss-of-function (antisense abrogation manoeuvres) coupled to classical pharmacological profiling has demonstrated for the first time a direct involvement of a specific tandem P-domain potassium channel (TASK3 in this case) in oxygen sensing by a cellular system [6].

Oxygen sensitivity of potassium channels is of crucial importance to our understanding of neuronal excitability. This is true not only in pathologies characterized by hypoxic episodes (such as global central ischemia following myocardial infarct or focal ischemic insult of cerebral vascular accident) but also in normal physiological function since cerebral partial pressure of O₂ (pO₂) is always very low compared to arterial levels. Here, we review some recent data which have demonstrated that recombinant human homologues of TASK1 and TREK1 demonstrate robust oxygen sensitivity

when expressed in HEK293 cells and discuss the potential impact such observations may have on the way we consider control of central neuronal excitability in health and disease.

Recombinant TREK1

The tandem P domain potassium channel TREK1 is localized almost exclusively to the central nervous system [8-11] although evidence also exists for its expression in the peripheral nervous system in general and sensory neurones of the dorsal root ganglia in particular [9]. Based on the ability of TREK to influence resting membrane potential of neurones, coupled to evidence of its specific regulation by unsaturated fatty acids [8, 12, 13], membrane stretch [12] and acidosis [9, 14], it has been suggested that this background K⁺ channel may control neuronal excitability and play a neuroprotective role during brain ischemia, where local pH declines and arachidonic acid is released into the extracellular space (see, for example, [15]). Further support for such an idea has come from the demonstration that the neuroprotective agent, riluzole, is a TREK1 activator [13, 16] and that arachidonic itself is neuroprotective [17]. Although this line of argument is compelling, it is based exclusively on studies of TREK1 channel regulation at pO₂ values of around 150 mmHg. Whilst this might be perfectly reasonable for potassium channels which are expressed in the periphery (since pO₂ is normally between 80 and 100 mmHg and O₂-sensitive K⁺ channels generally show little or no decline in activity between 150 and 80 mmHg (see, for example, [18]), it is clearly less pertinent in the CNS where ambient pO₂ has consistently been reported to be below 40 mmHg and may be as low as 20 mmHg in the human brain [19]. With this in mind, we recently studied the effect of a graded reduction in pO₂ on resting and stimulated activity of recombinant human TREK1 (hTREK1 - [20]). Like its murine homologue (mTREK1), hTREK1 is robustly activated by arachidonic acid (Fig. 1A) and membrane distortion using chemical crenators (Fig. 1B) and cup-formers (Fig. 1C). However, reduction in pO₂ to 60 mmHg and below evokes sustained and significant channel inhibition (Fig. 1D, E). Most importantly, hypoxia completely reverses hTREK1

Fig. 1 Recombinant hTREK-1 is O_2^- sensitive when expressed in HEK293 cells. **A.** Whole-cell currents evoked by the ramp-step protocol (shown at the lower part of D) during perfusion of normoxic control solution (C), in the presence of 10 μ M arachidonic acid (AA), and during washout (W) as indicated. **B.** As (A), except that the cell was exposed to perfusate containing trinitrophenol (TNP - 400 μ M) rather than AA. **C.** As (A) except that the cell was exposed to perfusate containing chlorpromazine (CPZ - 10 μ M) rather than AA. **D.** Exemplar whole-cell currents evoked by the ramp-step protocol shown before (C), during (H) and after (W) exposure to hypoxic solution, in hTREK-1 transfected HEK293 cells (upper traces) and wild type HEK293 cells (lower traces). Shown below these traces is the ramp-step protocol used to evoke all currents illustrated. **E.** Plot of normalized current amplitudes (I/I_{max}) versus chamber pO_2 levels (on a $\log_{10}$ scale). At each pO_2 level examined, the fractional inhibition (observed during a hypoxic challenge from 150 mmHg to the pO_2 level shown) is plotted for individual cells (open circles), and the mean effect is indicated by the horizontal bars.

channel activation by either arachidonic acid (Fig. 2A, B, C) or membrane stretch (Fig. 2 D, E). Furthermore, when the order of modulator addition is reversed, hypoxia completely occludes any effect of arachidonic acid [20]. These effects are mirrored using cell-attached patches [20].

As well as release of arachidonic acid, another key pathological feature of central ischemia is intracellular acidosis. hTREK1 channels are robustly activated by manoeuvres which evoke intracellular acidification (Fig. 3A, B, C) [21]. However, and consistent with the assumption that the mechanisms responsible for channel modulation by acidification, fatty acids and O_2 may demonstrate significant overlapping structural requirements, acid activation is completely occlud-

ed at low pO_2 (Fig. 3A, B, C) [21]. Furthermore, unlike its murine counterpart, hTREK1 is inhibited by intracellular alkalinization and neither arachidonic acid nor hypoxia are able to reverse this reduction in channel activity which is evoked by a rise in pH (Fig. 3 D, E, F). This leads us to the conclusion that there must be additional determinants required for channel closure during intracellular alkalosis [21]. As a consequence of these observations, it appears certain that up-regulation of hTREK1 activity (by either fatty acid or acidotic insult) during ischemia will not occur in the brain. Indeed, these data suggest that an inhibition of hTREK1 activity during situations of alkalosis is likely to be more physiologically relevant in tissues where ambient pO_2 is low.

Fig. 2 Hypoxia occludes arachidonic acid and TNP activation of hTREK-1. **A.** Typical time-series plot of current amplitude measured at +60mV showing the effects of hypoxia, and AA (10 μ M) in the presence and absence of hypoxia (~20mmHg). Periods of exposure to AA and hypoxic perfusate indicated by the horizontal bars. Numbered current traces in the inset correspond to the numbered points on the time series. **B.** Mean, normalized current amplitudes recorded at +60mV in control (normoxic) solutions and in the conditions indicated beneath each bar. **C.** Concentration-response curves for AA-evoked, normalized whole-cell currents under normoxic ($\square$) and hypoxic ($\blacksquare$) conditions. Each point plots mean, normalized current enhancement due to exposure to varying AA concentrations. Dotted line indicates the mean normalized current amplitude evoked by hypoxia alone (taken from B). **D.** Typical time-series plot of current amplitude measured at +60mV showing the effects of trinitrophenol (TNP) in normoxia, and the complete reversal of its effects by hypoxia. Periods of exposure to TNP and hypoxic perfusate indicated by the horizontal bars. Numbered current traces in the inset correspond to the numbered points on the time series. **E.** Mean, normalized current amplitudes recorded at +60mV in control (normoxic) solutions and in the conditions indicated beneath each bar.

Recombinant TASK1

Depression of activity of TASK-like potassium channels has been suggested to be central to the hypoxic response of arterial chemoreceptors, airway chemoreceptors and cerebellar granule neurons [22]. Consistent with earlier observations [23], recombinant hTASK1 channels stably expressed in HEK293 cells are acutely and reversibly modulated by altering extracellular pH (pH_o) (Fig. 4A, B) [24]. Raising pH_o from 7.4 elicits significant augmentation of currents whilst decreasing pH from 7.4

results in current reduction (Fig. 4B). Untransfected cells typically display no pH-sensitive currents. All cells expressing this signature pH-sensitivity are sensitive to changes in pO₂. At pH_o of 7.4, reduction of pO₂ to <40mmHg results in rapid and reversible suppression of pH-sensitive K⁺ currents (Fig. 4C). Furthermore, these two regulatory signals clearly act at the same channel, since the magnitude of the O₂-sensitive current is dependent on pH_o. Thus, lowering pH_o from 8.4 to 5.4 significantly reduces the hypoxic response (Fig. 4D). These data represent the first direct verification that hTASK-1 is an O₂-sensitive K⁺ channel, and rein-

Fig. 3 Modulation of the hTREK1 pH responses by hypoxia. **A.** Intracellular acidification: Typical whole cell hTREK1 currents evoked by the ramp-step protocol during perfusion with the agents defined at the right of the current traces. **B.** Exemplar time course of the effect of intracellular acidification with 10mM propionate (horizontal bar) before and during bath hypoxia (hatched area) on normalized hTREK1 currents. **C.** Bar graph showing mean, normalized current densities under the conditions indicated to the foot of the graph. **D.** Intracellular alkalinization: Typical whole cell hTREK1 currents evoked by the ramp-step protocol during perfusion with the agents defined at the right of the current traces (#1 and #2 indicate the first and second TMA-alone conditions, respectively). **E.** Exemplar time course of the effect of intracellular alkalinization with 20mM TMA (horizontal bar) before and during bath hypoxia (hatched area) on normalized hTREK1 currents. **F.** Bar graph showing mean, normalized current densities under the conditions indicated to the foot of the graph. Open bars represent TMA, solid bars represent ammonium.

force the notion that this channel is key to signal transduction in O_2 sensing tissues [6].

Potential physiological roles

TREK1 and TASK1 demonstrate essentially non-overlapping distribution patterns in the brain with the highest expression of TREK1 mRNA in the hippocampus, striatum and parts of the cortex and TASK1 mRNA expression localized in cerebellum and somatic motoneurons [25]. Functional expression of TASK1 has been demonstrated in isolated cerebellar granule neurons (where it underlies the

standing outward current of these cells [3]) and hypoxic suppression of the standing outward current results in increased excitability [22]. Thus, during ischemia, the hypoxic cue would exert an inhibitory influence on TASK1 and, as a consequence, increase excitability. Similarly, TREK1 expression in hippocampal neurons contributes to their resting membrane potential and changes in its activity result in dynamic changes in excitability [26]. Thus, during ischemia, TREK1 inhibition would lead to increased hippocampal neuronal excitability and may represent a contributory factor in the excitotoxicity which often ensues following ischemic insult. Activation of TREK1 during ischemia has previously been suggested to be neu-

Fig. 4 Interaction between pH and hypoxia on activity of hTASK1. **A.** Example time series plot (current measured at 0mV from a ramp). Periods of exposure to perfusates of different pH_0 levels indicated on top-right axis. **B.** Normalized, mean time series plot of the effect of hypoxia ($\text{pO}_2 = 25\text{-}30$ mmHg) on currents measured at 0mV at a pH_0 of 7.4. Period of exposure to hypoxic perfusate is indicated by the horizontal bar. **C.** Typical time-series plot showing the effect of hypoxia in a cell perfused with bath solution at pH_0 values indicated on the top-right axis. Periods of exposure to hypoxic perfusate is indicated by the horizontal bar. **D.** Mean inhibition of hypoxia (as a proportion of the total current measured in $\text{pH}_0 = 7.4$) plotted against pH_0 , demonstrating interactivity of these two modulators.

roprotective. However, since the action of all known TREK1 modulators, intracellular alkalosis notwithstanding, are without effect in low pO_2 [21], neuroprotection can clearly not be afforded by TREK1 activation during ischemia. Moreover, the non-overlapping requirements for oxygen- and alkali-sensing suggest that TREK1 may be more intimately involved in controlling excitability during alkalosis [21]. It is tempting to speculate, therefore, that in CNS neurons where TREK1 expression is a major influence on resting membrane potential, respiratory or metabolic alkalosis will cause hyperexcitability. Alkalosis has several effects on the CNS but, in general, it evokes increased activity often leading to aberrant afferent and efferent firing. Although regulation of hTREK1 activity is unlikely to account fully for such phenomena, it

may account for a component of such physiological responses. Equally, respiratory alkalosis is a characteristic of rising to high altitude as carotid body hypoxic drive to the medullary respiratory centers promotes hyperventilation and, therefore, removal of CO_2 . Under such circumstances, hyperexcitability of descending inhibitory neurons to the respiratory centre may temper the hyperventilation and prevent further alkalosis during a sustained visit to altitude. If true, TREK1 modulation may be involved in the acclimatization response. In this regard, it is interesting to note that hTREK1 predominately co-localizes with GABAergic neuronal markers in inhibitory interneurons [27], suggesting that TREK1 may be involved in controlling excitability via removal of tonic inhibitory influences during alkalosis.

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