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Brief Review

Translating blood-borne stimuli: chemotransduction in the carotid body

Prem KUMAR B. Sc.*, D. Phil

Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TT, UK

Abstract: The carotid body can transduce hypoxia and other blood-borne stimuli, perhaps including hypoglycaemia, into afferent neural discharge that is graded for intensity and which forms the afferent limb of a cardiorespiratory and neuroendocrine reflex loop. Hypoxia inhibits a variety of K^+ channels in the type I cells of the carotid body, in a seemingly species-dependent manner, and the resultant membrane depolarisation is sufficient to activate voltage-gated Ca^{2+} entry leading to neurosecretion and afferent discharge. The ion channels that respond to hypoxia appear to do so indirectly and recent work has therefore focussed upon identification of other proteins in the type I cells of the carotid body that may play key roles in the oxygen sensing process. Whilst a role for mitochondrial and/or NADPH-derived reactive oxygen species (ROS) has been proposed, the evidence for their signalling hypoxia in the carotid body is presently less than compelling and two alternate hypotheses are currently being tested further. The first implicates haemoxygenase 2 (HO-2), which may control specific K^+ channel activation through O_2-dependent production of the signalling molecule, carbon monoxide. The second hypothesis suggests a role for the cellular energy sensor, AMP-activated protein kinase (AMPK), which can inhibit type I cell K^+ channels and increase afferent discharge when activated by hypoxia-induced elevations in the AMP:ATP ratio. The apparent richness of O_2-sensitive K^+ channels and sensor mechanisms within this organ may indicate a redundancy system for this vital cellular process or it may be that each protein contributes differently to the overall response, for example, with different O_2 affinities. The mechanism by which low glucose is sensed is not yet known, but recent evidence suggests that it is not via closure of K^+ channels, unlike the hypoxia transduction process.

Key words: carotid body; chemoreceptor; hypoxia; chemotransduction; AMP-activated protein kinase; glucose

颈动脉体对化学信号的换能作用

Prem KUMAR B. Sc.*, D. Phil

伯明翰大学医学院生理学系，伯明翰 B15 2TT，英国

摘 要：颈动脉体可以将低氧和血液中其它刺激信号(可能包括低血糖)转换成不同强度的传入神经放电，沿心肺和神经内分泌反射的传入途径进入中枢，形成反射环路。低氧可抑制颈动脉体Ⅰ型细胞中的多种 K^+ 通道，这种作用可能有种属差异；K^+ 通道的抑制使膜电位去极化，启动电压依赖性 Ca^{2+} 内流，最后导致神经分泌和传入放电。离子通道对低氧的反应可能是通过间接途径发生的，因此近期的工作集中在研究颈动脉体Ⅰ型细胞中在低氧感受中起关键作用的其它蛋白质。虽然有人认为来源于线粒体和/或 NADPH 的活性氧(reactive oxygen species, ROS)起一定作用，但是它们在颈动脉体中转导低氧信号的证据还不足。目前正在对另外两种假设进行检验。第一种假设是血红素加氧酶 2 (haemoxygenase 2, HO-2)通过信号分子 CO 控制特殊 K^+ 通道的活动，而 CO 的生成量与氧分压高低有关。第二种假设是认为细胞能量感受器腺苷酸活化蛋白激酶(AMP-activated protein kinase, AMPK)起作用；低氧时 AMP/ATP 比值升高，激活 AMPK，从而抑制Ⅰ型细胞的 K^+ 通道，传入放电增加。颈动脉体的细胞上具有丰富的对氧敏感的 K^+ 通道，低氧感受这个重要的细胞活动可以通过多条途径进行，在总反应中每种蛋白质也可能起不同的作用，例如不同蛋白质对氧的亲合力不同等。关于颈动脉体感受低血糖的机制尚不清楚，但最近有证据提示，它并非由 K^+ 通道关闭引起的，因此感受低血糖的机制和感受低氧的机制是不同的。

关键词：颈动脉体；化学受体；低氧；化学转换；腺苷酸活化蛋白激酶；葡萄糖
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*Corresponding author. Tel: +44-121-4146907/4146910; Fax: +44-121-4146919; E-mail: p.kumar@bham.ac.uk

Introduction

The precise partial pressure of O_2 (PO_2) in any particular mammalian tissue depends on a number of factors, including the vascular structure of the perfused tissue, its cellular O_2 consumption rate and local diffusion conditions, but appears to be maintained within a relatively narrow range of 40-50 mmHg (7.5 mmHg = 1 kPa). This value can decrease during increased metabolism[1] if not matched by a compensatory increase in blood flow and if this mismatch is sustained over many hours, compensatory modifications in protein expression occur[2]. Within a timescale of seconds to minutes, however, the delivery of sufficient O_2 to meet metabolic demand will depend, ultimately, on augmented alveolar ventilation and redirection of blood flow. This acute response requires sensory systems that rapidly detect falls in arterial oxygenation, coupling this to reflex and/or hormonal cardiorespiratory responses. The major sensor of acute arterial hypoxia is the carotid body peripheral chemoreceptors, which, together with pulmonary artery smooth muscle cells, kidney erythropoietin secreting cells and possibly airway neuroepithelial bodies, act to ensure systemic protection from the damaging effects of hypoxia. This brief review gives an account of current hypotheses of O_2 transduction in the carotid body and suggests that it may be profitable to consider the carotid body rather as a poly-modal stress sensor than as a hypoxia sensor *per se*.

Role of K^+ channels

At rest, the carotid bodies contribute 10%-20% of the total ventilatory drive but this proportion increases as their discharge increases sharply when arterial PO_2 falls below *ca*. 60 mmHg. Detection of hypoxia by the carotid body occurs in the type I cells of this organ. These cells are approximately 10 μm in diameter and are arranged in clusters, surrounded by glial-like type II cells and in close apposition to both free nerve endings of the afferent carotid sinus nerve branch of the glossopharyngeal nerve and a rich blood supply. Since the late 1980s it has been recognised that the type I cells can respond to hypoxia by inactivation of a variety of plasmalemmal K^+ channels thus increasing membrane resistance and inducing sufficient cell depolarisation to activate voltage-gated Ca^{2+} entry. The elevation in intracellular Ca^{2+} leads to neurosecretion of a variety of neuromodulator and transmitter substances from the type I cells and this generates action potentials in the post-synaptic carotid sinus nerve afferents[3]. A variety of K^+ channels with O_2 sensitivity have been described including the classical voltage-dependent K^+ channels[4], non-inactivating Ca^{2+}-dependent K^+ channels, particularly BK[5] channels, hERG K^+ channels[6] and subunits of the voltage-independent, acid- sensitive tandem P domain K^+ (TASK) channel family[7]. Species differences do exist but these channels may act in concert within a single cell to both induce and maintain the depolarisation. O_2-sensitive K^+ channel in the type I cells is most often observed only in those patch clamp configurations that retain cytosolic or closely-associated factors, e.g., whole-cell or perforated patch recordings[8,9], which suggests that the channel itself is not O_2-sensitive but is, instead, coupled directly or indirectly to a non-membrane delimited sensor located elsewhere within the cell.

Sensors of hypoxia

One proposed mechanism assumes a key role for gas molecule signalling in the inactivation of Ca^{2+}-dependent K^+ channels as the O_2-dependent enzyme, haemoxygenase-2 (HO-2) was found to be closely associated in a protein complex with membrane bound K^+ channels and its product, carbon monoxide (CO), appeared essential for BK channel activation[10]. Thus, in the presence of O_2 and its co-substrates, haem and NADPH, CO increases the channel open probability and when O_2 levels are reduced in hypoxia, the reduction in CO production would inactivate the channel leading to membrane depolarisation. This is an intriguing hypothesis, but the importance of HO-2 has been questioned recently by the finding that HO-2 null mice appear to have unchanged hypoxia sensitivity despite alterations in BK channel expression[11]. Reconciling these differences will be an important next step in the development of this hypothesis.

Other hypotheses for O_2 sensing are based upon global changes in the cellular redox or energy status. These hypotheses have their origin in the finding that blockers of the mitochondrial electron transport chain or uncouplers of oxidative phosphorylation are potent chemostimuli. A role for mitochondrial proteins in O_2 sensing has, therefore long been suggested, but requires type I cell mitochondria to possess some, as yet undefined, specialisation that confers upon them a physiological sensitivity to hypoxia[12,13]. It has, however, been reported that many inhibitors of oxidative phosphorylation may have non-specific actions upon K^+ channel activity that are independent of the effects of hypoxia[14] and, coupled with the finding that partial deletion of *sdhd*, a component of mitochondrial complex II, in mice had no effect upon hypoxia sensitivity as assayed by catecholamine release from the type I cells[15] means that a role for mitochondria in O_2 sensing is not yet fully established.

That said, current mechanisms to account for the coupling of mitochondria with the K^+ channels of the plasma membrane include variations in the concentration of reactive oxygen species (ROS), acting via changes in cellular redox potential. Although mitochondrial ROS may affect K^+ channel function, their role in O_2 sensing is contentious[16]. Another source of ROS localised more closely to plasmalemmal K^+ channels is the extra-mitochondrial membrane bound NADPH oxidase complex, which produces O_2^- and H_2O_2, in proportion to PO_2[17]. Unfortunately, targeted deletion of key NADPH oxidase subunits, including gp91phox[18,19] and p47phox[20] does not reduce O_2 sensitivity, but instead suggestes that ROS may be involved in cell repolarisation rather than depolarisation.

Alternatively mitochondria may couple to K^+ channel inhibition via changes in the cytosolic concentration of ATP or factor(s) related to ATP synthesis. Although a direct action of ATP upon TASK channels has been described in isolated membrane patches[21], it seems likely that this occurs indirectly in the whole cell. One such mechanism is via hypoxia-mediated increases in the cellular AMP:ATP ratio, raising AMP levels sufficiently to stimulate *de novo* synthesis of AMP-activated protein kinase (AMPK)[22]. AMPK is ubiquitously expressed in eukaryote cells and plays a vital role in cellular metabolic regulation in a variety of tissues[23] by augmenting ATP generating processes whilst inhibiting non-essential energy consuming processes. Recently, both Ca^{2+}-dependent K^+ channels and TASK channels of the carotid body type I cells, have been shown to be novel targets of this kinase and are inactivated by the AMPK mimetic agent, 5-aminoimidazole-4-carboxamide riboside (AICAR)[24]. This effect is sufficient to enable Ca^{2+}-dependent neurosecretion and an augmented carotid body afferent discharge and, crucially, the AMPK antagonist, compound C, can prevent hypoxia-mediated excitation. This hypothesis is particularly compelling as it unites the mitochondrial and K^+ channel responses to hypoxia without a need to necessarily invoke mitochondrial specialisation or decreases in the concentration of ATP. This latter point is particularly important for the energy consuming process of chemotransduction. An implication, however, if one assumes that the type I cells have a sensitivity to hypoxia not observed in other non-O_2 sensing tissues, must be that the carotid body either possesses unique isoform complexes of AMPK or that signalling by AMPK is somehow modified for specific function in these cells.

Sensing glucose: an alternate or complementary function?

As described above, the carotid body can respond to hypoxia and much of the research focus in recent years has been focussed upon elucidating the transduction mechanism for this stimulus. The carotid body is, however, a poly-modal receptor and has a number of adequate stimuli. Thus, in addition, to hypoxia, it has the ability to sense a variety of other stimuli including blood CO_2, pH, temperature, osmolarity and hyperkalaemia, as well as hypotension, transducing them into afferent discharge at physiological levels of intensity. Interactions between these various stimuli and hypoxia are known to exist. More recently, a novel role for the carotid body as a glucosensor has been proposed following initial reports that intracarotid glucose infusion could reduce carotid chemoreceptor activity and carotid body stimulation could increase hepatic glucose output and brain glucose retention[25]. Additionally, chemo-denevated dogs failed to mount an adequate, counter-regulatory neuroendocrine response when challenged with an insulin-mediated hypoglycaemia[26], although the stimulus involved in this study has been suggested to be an, as yet, unidentified product of metabolism rather than hypoglycaemia *per se*[27] that acts to augment CO_2 sensitivity[28]. A direct glucosensor role has, however, been indicated in *in vitro* studies. Thus, in a novel thin slice preparation of the carotid body, low glucose (< 2 mmol/L), like hypoxia, stimulated catecholamine secretion[29,30] and could also excite post-synaptic petrosal neurons in a co-culture of petrosal neurons and type I cells[31]. However, reductions of superfusate glucose to 2 mmol/L or less in whole organ preparations of the carotid body had no acute effect upon chemodischarge[27,32], and a definitive direct role for the carotid body in glucose sensing has not yet been established. The reason for the discrepancy between the various reduced models is not yet known, although it has been suggested that it may be to do with concomitant levels of O_2[33,34] with hyperoxia, in some way, reducing or preventing a hypoglycaemic response. More work is required before the carotid body can definitely be placed into a list of peripheral glucosensor tissues that include the pancreas and liver. Whatever the outcome, an interesting finding has been that low glucose reduces type I cell input resistance, indicating channel opening[34]. The precise channels opened are not known but it is clear that this is different from the inhibitory effect of hypoxia upon ion channels. This significant difference in mechanism could be taken to suggest separate transduction pathways for glucose and hypoxia sensing which may be important for an organ that requires an increase in its own metabolic rate at a time of reduced O_2. The implications of any hyperoxia-mediated

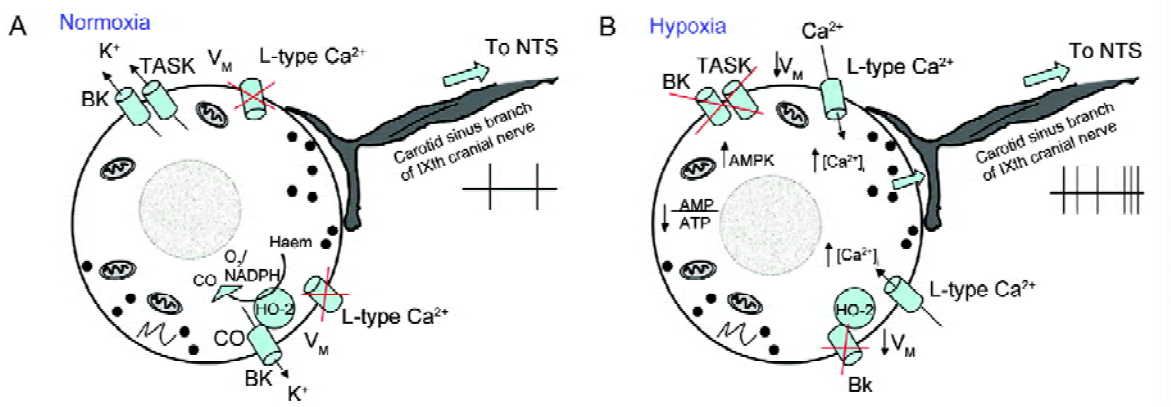

Fig. 1. Schematic representation of hypoxia sensing mechanisms in the carotid body type I cell. *A*: During normoxia, the resting membrane potential (V_M) of the type I cell is maintained by efflux of K^+ ions through a variety of K^+ channels including the large conductance Ca^{2+}-activated K^+ (BK) channels and specific subtypes of the acid-sensitive tandem P domain K^+ (TASK) channel family. TASK channels are voltage-independent and are open at the resting V_M whilst, in the presence of O_2, the enzymic activity of the BK-associated protein, haemoxygenase 2 (HO-2), generates carbon monoxide (CO) that acts to activate the BK channels. At the resting V_M, L-type voltage-gated Ca^{2+} (L-type Ca^{2+}) channels are inactivated and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and therefore neurosecretion and afferent action potential frequency are basal. *B*: In reduced O_2 tensions (hypoxia), the V_M of the type I cell is reduced with this depolarisation due to an inactivation of TASK and BK channels. The inactivation of TASK and BK channels is associated with an elevation in the cytosolic concentration of AMP-activated protein kinase (AMPK) that is generated by an increase in the cellular AMP/ATP ratio occurring as a consequence of the reduced O_2. In addition, the CO-mediated activation of BK channels is removed. The depolarisation is of sufficient magnitude to induce an elevated $[Ca^{2+}]_i$ via Ca^{2+} influx through L-type Ca^{2+} channels. Neurosecretion and afferent action potential frequency are elevated leading to a variety of corrective cardiorespiratory and autonomic reflexes.

inhibition of glucose sensing have yet to be appreciated but, on the face of it, appear similar to the reducing effect of high O_2 upon CO_2 sensitivity[35,36].

Concluding remarks

The carotid body transduces arterial hypoxia into graded afferent neural output. Recently, novel models for this process have been described that now require further testing with more selective experimentation. Determination of more than one sensor protein and a number of ion channels that can be modulated by hypoxia (Fig.1) may be taken to indicate the importance of this process and/or it may be that each sensor system has its own particular O_2 affinity and that only when combined, does the whole, *in vivo* stimulus-response characteristics become apparent. The potential for redundancy in such a vital process may make interpretation of single gene deletion experiments difficult but these are beginning to provide data that needs to be incorporated into the current hypotheses of O_2 sensing. In addition, the organ may also detect falls in blood glucose, possibly giving it a key role in sensing body energy, or metabolic status and initiating vital counter-regulatory, reflex and humoural responses. Interactions between stimuli may increase the afferent discharge during a variety of physiological and/or pathophysiological situations, including exercise and haemorrhage and act to ensure appropriate cardiorespiratory and neuroendocrine reflex compensation in time of stress.

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