CHAPTER 4

ELECTRICAL PROPERTIES OF CHEMORECEPTOR CELLS

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INTRODUCTION

Carotid body (CB) chemoreceptor cells, in spite of their neural origin, were considered nonexcitable until the late 1980's. The remarkable complexity of the organ, together with the small size of type I cells, represented a limitation for conventional intracellular microelectrode recordings, making a definitive electrophysiological study problematic. The neurochemical approach used during the early 1980's, following the stimulus-secretion model established in other neurosecretory systems, suggested an important role for the plasma membrane of type I cells in the hypoxic chemotransduction process. Development of isolated type I cell cultures, together with the use of the patch-clamp technique, have brought direct evidence in support of this idea.[1,2] We now have a general picture about the electrical properties of these cells, and their excitable character is unequivocally established; they possess voltage-dependent ion channels and they are capable of firing action potentials. Although there is a general agreement in the literature about the basic facts, the details are far from being clear. The role of ionic currents in the transduction process by type I cells has been a matter of discussion, and differences in the results reported by different laboratories are evident. In most of the cases the differences could be interpreted on basis of the fact that either cells from different species or at different stages of development have been studied, but in some cases, the differences have led to the proposal of different hypotheses about the mechanisms of chemotransduction.

This chapter will present an overview of the ionic currents present in type I cells, dwelling especially on those differences. All patch-clamp data available so far have been obtained from rabbit or rat type I cells isolated either from adult or neonatal animals. When physiological intra- and extracellular solutions are used, chemoreceptor cells exhibit, on step depolarization, inward currents due to Na^+ and/or Ca^{2+} channels, and outward currents due to

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multiple types of K^+ channels. Below, we consider the separate ionic currents of type I cells, and compare results obtained from different preparations in different laboratories.

NA^+ CURRENTS

Using standard procedures for blocking K^+ currents (i.e., intracellular Cs^+) and Ca^{2+} currents (i.e., extracellular Cd^{2+}), Na^+ currents (I_{Na}) can be routinely recorded in isolation in adult rabbit type I cells[3,4] (Fig. 4.1A). I_{Na} is sensitive to tetrodotoxin (TTX), has an apparent activation threshold around -40 mV and a fast activation time course, reaching its peak in less than 1 ms. The peak amplitude at 0 mV is approximately 0.4 nA on average. The half steady-state inactivation occurs at a membrane potential of -50 mV. Inactivation follows a monoexponential time course, with a time constant of 0.67 ms at 0 mV. All these characteristics are typical of Na^+ currents found in other neurosecretory cells.

Na^+ currents have not been found in rabbit embryos[5] and their presence is not ubiquitously reported in rat type I cells. Some groups have reported a lack of Na^+ currents in freshly dissociated type I cells from adult[6] and young rats.[7] However, other groups have reported rapidly activating and inactivating TTX-sensitive Na^+ currents present in cells from young rats maintained in long-term cultures[8] or in freshly dissociated cells from adult[9] and neonatal rats.[10] The wide range of reported percentages of cells having Na^+ currents in rat cells (from none[6,7] to 46% [10]) may reflect age-related differences or more

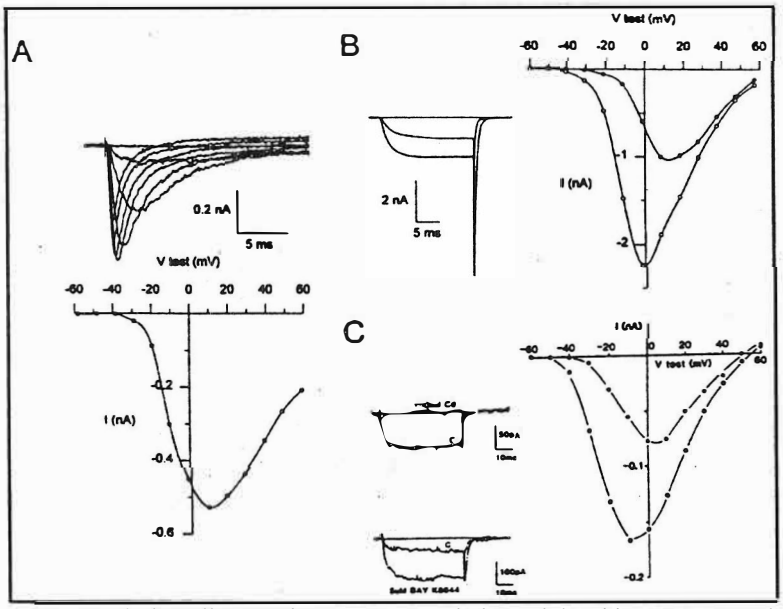

Fig. 4.1. Whole-cell inward currents recorded in adult rabbit (A & B) and neonatal rat (C) cells. (A) Family of Na^+ currents recorded in the presence of 0.1 mM Cd^{2+}. The I-V relationship of the peak current is also shown. (B) I-V relationship for Ca^{2+} currents obtained in an adult rabbit chemoreceptor cell using 10 mM Ca^{2+} (filled circles) or 10 mM Ba^{2+} (open circles) as charge carriers. Na^+ currents are blocked with 0.1 mM TTX. The currents obtained with Ca^{2+} and Ba^{2+} at +10 mV are shown superimposed. (C) Ca^{2+} channel currents recorded in a neonatal rat type I cell using 10 mM Ba2+ as charge carrier. Note the lack of rapidly activating and inactivating Na^+ current, despite the fact that TTX was not present. Ca^{2+} channel currents could be fully blocked by 100 mM Cd2+ and enhanced by the DHP agonist Bay K 8644 (5 mM). The I-V relationship from the same cell is shown in the absence (filled circles) and presence (open circles) of Bay K 8644.

likely, an heterogeneous expression of Na^+ channels in type I cells, as has been proposed with respect to the different types of Ca^{2+} channels (see below).

CA^{2+} CURRENTS

Neurochemical studies of dopamine release from intact carotid bodies implicated L-type (dihydropyridine (DHP)-sensitive) Ca^{2+} channels as being involved in hypoxic chemotransduction before their presence were confirmed with the patch-clamp technique.[11,12] Typically, Ca^{2+} currents (I_{Ca}) can be recorded in isolated type I cells when K^+ currents are blocked with internal Cs^+, and TTX is present in the bath solution. I_{Ca} is present in both rabbit and rat cells, and it appears to be mainly due to L-type Ca^{2+} channels (Figs. 4.1B and C).

Kinetic properties of I_{Ca} have been studied thoroughly in rabbit cells using both Ca^{2+} and Ba^{2+} as charge carrier[3,4] (Fig. 4.1B). The apparent activation threshold is about -40 mV, and the peak current is obtained at +10 to +20 mV. I_{Ca} inactivation is very slow, and 200 ms after the onset of depolarization the current amplitude is still about 70% of the peak current. When I_{Ca} is elicited with a depolarizing pulse, the relatively small current is followed by a much larger inward tail current, the time course of which reflects the closing of Ca^{2+} channels and has been used to define the type of Ca^{2+} channels present in type I cells. Tail currents can be fitted by the sum of two exponential functions, suggesting that I_{Ca} is carried through more than one type of Ca^{2+} channel. The biggest component has a time constant typical of the fast deactivating or L-type currents (160 ms). The other component (20 times smaller) could reflect the slow deactivating or T-type current, although its presence has not been confirmed using different voltage protocols. I_{Ca} shows also the phenomenon of wash out or run down. Wash out is use- and ATP-dependent, as happens typically with L-type Ca^{2+} currents.[3,4]

In the last few years the classification of Ca^{2+} channels in different cells has been made mainly through pharmacological criteria, using different peptide toxins. At present, there is little available evidence in the literature concerning the use of toxins to characterize I_{Ca} in rabbit type I cells further. However, a recent brief report[13] has indicated that I_{Ca} in adult rabbit type I cells is sensitive to w-conotoxin GVIA and to w-agatoxin IVA as well as to nifedipine, indicating the possible presence, in addition to L-type channels, of N- and P-type. This finding supports the idea that multiple Ca^{2+} channel types coexist in adult rabbit type I cells.

Rabbit embryo type I cells also have Ca^{2+} currents with properties similar to those of the adult animals, which are activated by the DHP agonist Bay K8644 and blocked by D600, as expected for L-type Ca^{2+} channels.[5] However, blockade of I_{Ca} is incomplete even when high (10 mM) concentrations of organic blockers are used, which may suggest that these cells also possess non-L-type Ca^{2+} channels.[5]

Whole-cell patch-clamp recordings from neonatal (as well as adult) rat type I cells have confirmed the presence of L-type channels, since whole-cell Ca^{2+} channel currents can be enhanced by Bay K 8644, and can be suppressed by DHP blockers[7,14,15] (Fig. 4.1C). However, as for adult rabbit cells (see above), L-type channels do not account for all of the Ca^{2+} channel current: maximal or supramaximal concentrations of DHP blockers do not completely inhibit Ca^{2+} currents,[15-17] nor do they fully prevent the voltage-dependent rise of $[Ca^{2+}]_i$ seen in response to high K^+-containing solutions.[18,19] The remaining, non-L-type current has yet to be fully characterized, but there is evidence for a heterogeneous distribution of N-type channels in some type I cells from young and adult rats. Fieber and McCleskey found the N-type channel blocker w-conotoxin GVIA (w-CgTx) to partially inhibit currents in one of four cells,[6] and studies in 10 day old rat type I cells have shown partial inhibition by w-CgTx in two out of eight cells.[16] Stea et al reported a lack of effect of w-CgTx,[14] but its effects were only tested in four cells. The possibility that N-type channels exist in some but not all type I cells would suggest that the cells themselves are heterogeneous (although Silva and Lewis have reported a 40% inhibition of peak Ca^{2+}

current by w-CgTx in all the cells they have tested, when recording in type I cells from adult rats).[15] These findings also suggest that type I cells from young and also adult rats possess a type or types of Ca^{2+} channel which are not L- or N-type.

Despite the similar, heterogenous nature of Ca^{2+} channels in rabbit and rat type I cells, the difference in size of whole cell currents is quite remarkable. Using Ba^{2+} as the charge carrier, the peak amplitude is about 50 pA in rat type I cells, more than 10 times smaller than the Ca^{2+} currents in adult rabbit cells (typically ranging between 0.4 and 1 nA); if we take into account the fact that the size of the cells are similar, the density of channels per unit of membrane surface area has to be much bigger in the adult rabbit type I cells.

K^+ CURRENTS

When type I cells are dialyzed with a solution containing high $[K^+]$, and are perfused with a solution of physiological composition, the dominant currents elicited upon membrane depolarization are outward currents that exhibit a voltage dependence and a sensitivity to blockers such as tetraethylammonium (TEA) and 4-aminopyridine (4-AP), characteristic of K^+ currents (IK).[3-5,20,21]

IK from rabbit type I cells can be divided into at least three different components.[20] If IK is recorded in the absence of Ca^{2+} channels blockers, the current-voltage (I-V) relationship exhibits a typical outward shoulder at voltages between 0 and +40 mV (the potential range at which the Ca^{2+} current is predominant). This shoulder disappears either after Ca^{2+} channel currents wash out or after application of Ca^{2+} channel blockers (Fig. 4.2A). Therefore this component of IK is clearly due to Ca^{2+}-dependent K^+ channels, the presence of which has been confirmed using single channel recordings.[22] Although only a 210 pS Ca^{2+}-dependent K^+ channel (maxi-K or BK) has been recorded at the single channel level, the shoulder in the IK I-V relationship is also partially inhibited by apamin,[3] suggesting the presence of small conductance Ca^{2+}-dependent K^+ channels in rabbit cells.

When I_K is recorded after Ca^{2+} currents are washed out or in the presence of Ca^{2+}-channels blockers only the voltage dependent component of IK (IK_v) remains. IK_v inactivates during long step depolarizations, but the inactivation is not complete, sug-

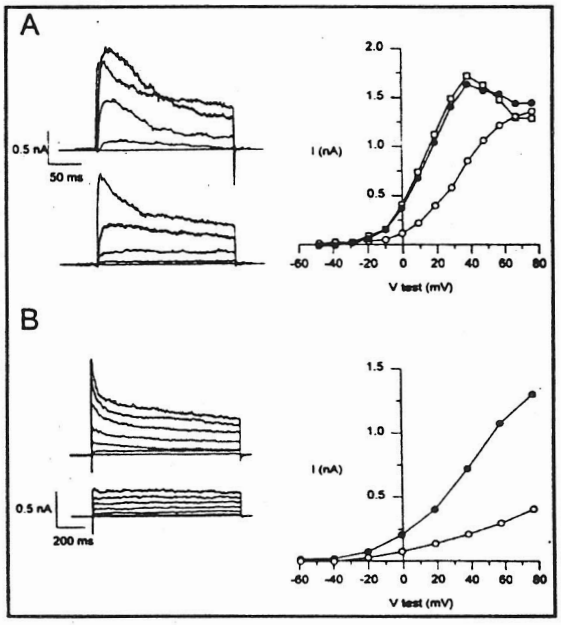

Fig. 4.2. Whole-cell K^+ currents recorded in adult rabbit chemoreceptor cells. (A) I-V relationship for IK obtained in solution control (open squares), in the presence of 1 mM Co^{2+} (open circles) and after washing the solution with Co^{2+} (filled circles). The typical shoulder in the I-V curve, due to the Ca^{2+}-dependent K^+ channels, is clearly inhibited by the Ca^{2+} channel blocker Co^{2+}. The currents shown are those elicited at -10, +10, +30 (thicker trace) and +70 mV, both in solution control (up) and in the presence of Co^{2+} (down). (B) I-V relationship of IKv obtained (after Ca^{2+} currents washed out) in a physiological solution (filled circles) and in the presence of 1 mM 4-AP. The original traces are also shown. Note how in the presence of 4-AP (down), the inactivating component of the current is not present.

gesting that more than one type of channel is contributing to IK_v. In fact, two different components can be isolated using 4-AP[20] (Fig. 4.2B). The inactivating component is fully removed by the application of 1 mM 4-AP (IC_{50} of 0.2 mM) and both the transient and the noninactivating components are sensitive to TEA at concentrations greater than 5 mM. The existence of these two components are confirmed when steady-state inactivation properties are studied. H• curves are well fitted with a Boltzman distribution plus a constant component (the noninactivating component) which only represents 10% of the total IK_v.[20] The apparent threshold for activation of IK_v is around -40 mV, and this is also the potential at which the transient current is 50% inactivated in the steady state. The time course of the inactivation is relatively slow, and it can be described by two exponential functions with time constants of 80 and 825 ms.[20] At the single channel level, two different voltage-dependent, Ca^{2+}-insensitive channels have been described from inside-out excised patches.[22] One of them, named SK, has a conductance of 16 pS and exhibits very slow activation and almost no inactivation at all. The other is the most frequently found; it has a conductance of 40 pS and shows activation and inactivation kinetics quite similar to those found in the transient current seen in whole-cell recordings. It has been call Ko_2, because is the only one that is inhibited by hypoxia in excised patches.[22,23]

Cells from rabbit embryos also have Ca^{2+}-dependent and voltage-dependent components, both of which are sensitive to TEA.[5] Whole cell IK has not been studied in detail, but a K^+ channel with a conductance of 137 pS has been recorded in the cell-attached configuration. This channel has an

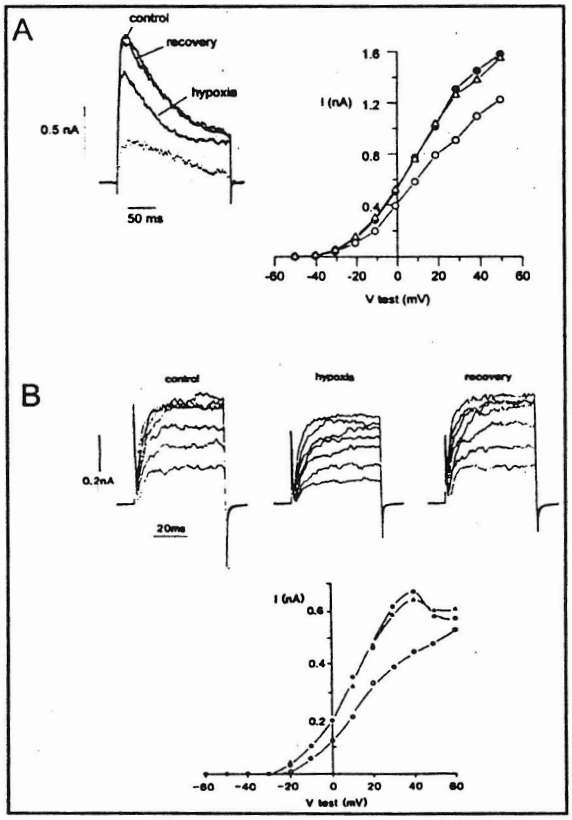

Fig. 4.3. Effects of hypoxia on whole-cell K^+ currents from adult rabbit (A) and neonatal rat (B) cells. I-V relationships of IK recorded in an adult rabbit (A) and in a neonatal rat (B) type I cell before (filled circles), during (open circles) and after (open triangles) exposure of the cell to hypoxia (ª5 mmHg in A, ª25 mmHg in B). The actual currents obtained at +40 mV as indicated in the rabbit cells, and the difference between control and hypoxia (dotted line) are also shown in A. The whole family of currents obtained in the rat cell are shown in B.

open probability that decreases with depolarization and that depends of the O_2 content of the bathing solution[24].

IK in neonatal rats have amplitudes of around 0.4 nA at +60 mV (although there is a wide cell-to-cell variability), which is much smaller than the typical values that are obtained in adult rabbit cells (typically in the range 1.5 to 3 nA) (compare Figs. 4.2A and 4.3B). The outward K^+ currents of neonatal rat type I cells have been shown to be sensitive to numerous pharmacological blockers including TEA, 4-AP and Ba^{2+} (see references 8 and 25). These currents have been divided into a Ca^{2+}-sensitive component and a voltage-gated, Ca^{2+}-insensitive component (IK_{Ca} and IK_v respectively). Since under the recording conditions most commonly used, $[Ca^{2+}]_i$ is not completely buffered, IK_{Ca} can be selectively inhibited with Ca^{2+} channel blockers,[7,21] because activation of K_{Ca} occurs during cell depolarization by Ca^{2+} influx through voltage-gated Ca^{2+} channels. For this reason, as in rabbit cells (see above), whole-cell I-V relationships also display an outward shoulder,[26] with amplitudes increasing steeply with depolarizations from *ca.* -30mV to +20mV, but then declining to varying degrees before once more increasing with membrane potential (Fig. 4.4B). Alternatively, IK_{Ca} can be directly inhibited by the scorpion venom peptide charybdotoxin (ChTx),[26] indicating that the underlying channels are high conductance (Maxi-K or BK) channels. More recently, this has been confirmed using single channel recording techniques which have revealed a channel of approximately 200pS conductance whose activity is steeply dependent on $[Ca^{2+}]_i$ (reference 27). Apamin has no effect in type I cells of young rats,[26] a finding which contrasts with its ability to partially inhibit K^+ currents in adult rabbit type I cells.[3] The Ca^{2+}-insensitive IK_v of rat type I cells has been less thoroughly studied than IK_{Ca}, but has been shown to have a linear current-voltage relationship, and to be sensitive to 4-AP.[28]

IK in adult rats have been recently characterized,[9] appearing to be very similar to IK in neonatal rats. Two components of IK, Ca^{2+} and voltage dependent (IK_{Ca} and IKv, respectively) are also evident, and as in neonatal rat chemoreceptor cells the ChTx sensitive current is the predominant. IK_v is a typical delayed rectifier, exhibiting a slow activation and a very slow inactivation, well described by the sum of two exponentials with t_1=0.68 and t_2=4.96 seconds.[9] Interestingly, the component of IK attributable to IK_{Ca} increases with age from 4 day old to 10 day old rats, but is similar in 10 day old and adult rat type I cells.[29] This may be of physiological importance, since IK_{Ca} is O_2 sensitive in rat cells (see below) and the maturation of O_2 sensitivity of the intact carotid body (as determined by carotid sinus nerve activity and catecholamine release.[30] is most evident up to 10 days of age.

EFFECTS OF ACUTE HYPOXIA ON IONIC CURRENTS

A major advancement in our understanding of carotid body chemotransduction came with the observation that hypoxia inhibits IK in type I cells. This was first reported in adult rabbit type I cells,[31] and has subsequently been shown to occur in other type I cell preparations. However, the component of IK sensitive to hypoxia is not the same in adult rabbit as compared with embryonic rabbit or rat cells.[8,9,21,24,32]

There are now numerous pieces of evidence from both whole cell and excised patch experiments supporting the view that the component of IK modulated by O_2 in rabbit cells is the transient component of IK_v (Fig. 4.3A). Low PO2 reversibly inhibits IK (IKv)[31,32] when Ca^{2+} channels are washed out (Fig. 4.3A), and in excised patches decreases the open probability of the inactivating K^+ channel (Ko_2).[22,23] The inhibition of the transient K^+ current increases the firing frequency of action potentials in type I cells,[32,33] which would produce an increase in Ca^{2+} entry to the cell through Ca^{2+} channels and an elevation of intracellular Ca^{2+} (references 11 and 34). The mechanisms of modulation of IK by O_2 are still a matter of controversy and there are different hypothesis in the literature. Based on the effect of hypoxia in isolated patches, it has been proposed that O_2 interacts with a membrane sensor directly coupled to Ko_2.[1,34] The nature of that

sensor is not known, but López-López and González have shown that CO prevents the hypoxic inhibition of IK, suggesting that the sensor could be a heme-linked protein present in the plasma membrane.[35] Other authors, in spite of the effect of low O_2 in isolated patches, have proposed that the sensor is a NAD(P)H oxidase complex, with some of their components located in the membrane and coupled to the K^+ channel through the generation of a second messenger (H_2O_2)[36] (see chapter 5 by Pérez-García and González)

The membrane model of hypoxic transduction, involving the modulation of cell excitability through the effects of O_2 in a K^+ channel, has been challenged by Biscoe and Duchen.[37] Using the same preparation of adult rabbit carotid bodies and cyanide (histotoxic hypoxia) as a stimulus,[38] they did not find an inhibition of IK, proposing that the effect of any type of hypoxia on ionic currents would be secondary to an elevation of intracellular Ca^{2+}. However, a lack of effect of hypoxia on IK has been also described occasionally in some preparations, and a good correlation exists between this fact and the total inability of the culture to release dopamine in response to hypoxic stimulation.[39]

The effect on whole cell K^+ currents is quite similar in rabbit embryos than in adult cells.[5,24] However, at the single channel level hypoxia decreases the open probability of a 137 pS channel. This channel has a different voltage dependence than the 40 pS channel inhibited in cells from adult animals (see above).[24] We lack a complete characterization of the single channel properties in these embryonic cells, and in order to compare their properties with those found in adult cells such characterization has to be done.

Hypoxia also inhibits K^+ currents in young.[8,21,28] or adult[9,29] rat type I cells. This effect has been shown to be selective for IK_{Ca} (Fig. 4.3B), although other workers have not determined whether or not hypoxia selectively inhibits a specific subtype of K^+ channel in their preparation. Stea and Nurse reported that hypoxia inhibited K^+ currents regardless of whether recordings were made using conventional whole-cell recordings or using perforated-patch recordings; (i.e., with or without dialysis of the cell interior).[8] This finding would suggest that hypoxic inhibition of K^+ channels did not depend on soluble cytosolic factors. However, no means of gauging the rate or extent of cell dialysis during conventional recordings were reported, so the possibile involvement of cytosolic mediators can not be fully discarded. More recently, single channel studies in young rat type I cells have shown that K_{Ca} channels are unaffected by hypoxia in conventional, excised outside-out patches and inhibition by hypoxia was only seen in perforated vesicle recordings where the channels remain in contact with a small volume of cell cytosol.[27] This finding opened up major discrepancies between findings in adult rabbit and young rat type I cells. In rabbit, hypoxia inhibits a specific Ca^{2+}-insensitive Ko_2 channel via a membrane-delimited mechanism.[22,23] In rat, hypoxic inhibition of K_{Ca} channels was dependent on as yet unidentified cytosolic factors.[27] Such differences are difficult to account for at present, but emerging in the literature is the awareness that O_2-sensitive channels are more widespread than in the carotid body,[40,41] and may not be confined to K^+ channel types, since O_2-sensitive L-type Ca^{2+} channels (both native and recombinant) have recently been documented.[42,43] The O_2 sensitivity of Ca^{2+} channels have been also reported in type I cells from adult rabbits,[33] although Ca^{2+} currents recorded from adult rat type I cells seem to be insensitive to hypoxia.[9] The role of O_2 modulation of Ca^{2+} channels in the chemotransduction process remains to be fully determined, but their voltage-dependent suppression by hypoxia may prevent excessive rises of $[Ca^{2+}]_i$ at inappropriately high PO_2 levels.

The role of IK_{Ca}, as the trigger of hypoxic depolarization required in the membrane model of hypoxic transduction in the rat, has been recently questioned by the observation that pharmacological inhibition of IK_{Ca} fails to stimulate either the intact carotid body[44-46] or isolated type I cells[47] under normoxic conditions. In this regard,

sensor is not known, but López-López and González have shown that CO prevents the hypoxic inhibition of IK, suggesting that the sensor could be a heme-linked protein present in the plasma membrane.[35] Other authors, in spite of the effect of low O_2 in isolated patches, have proposed that the sensor is a NAD(P)H oxidase complex, with some of their components located in the membrane and coupled to the K^+ channel through the generation of a second messenger (H_2O_2)[36] (see chapter 5 by Pérez-García and González)

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hypercapnia. In adult rabbit type I cells, this effect has been proposed to occur via Na^+ loading of type I cells (arising from increased activity of Na^+/H^+ exchangers) which leads to a reversal of Na^+/Ca^{2+} exchange, so that Ca^{2+} enters type I cells on this exchanger rather than through voltage-gated Ca^{2+} channels.[53] Indeed, although acidic stimuli inhibit IK in rabbit type I cells, there is a parallel reduction in I_{Na} and I_{Ca}[1], suggesting a nonselective inhibition of currents by hydrogen ions. By contrast, in young rat type I cells acidic transduction mechanisms are comparable with those for hypoxia.[7,26,54] Thus, a lowering of pH_o from 7.4 to 7.0 selectively inhibits K_{Ca} channels[26] and, importantly, this pH shift does not alter Ca^{2+} channel functioning.[7] The effects of pH_o are likely to be mediated via changes in pH_i, since selective intracellular acidosis (caused by application of salts of weak acids) also selectively inhibits K_{Ca} channels.[7] Furthermore, the exquisite sensitivity of the carotid body to acidosis may lie in the observation that, despite possessing numerous pH regulating mechanisms,[55] there is an extremely steep dependence of pH_i on pH_o.[56] In addition, Buckler and Vaughan-Jones have demonstrated that hypercapnic-induced rises of $[Ca^{2+}]_i$ are dependent on membrane depolarization, since voltage-clamping type I cells at their resting membrane potential prevented a 20% CO_2 stimulus from raising $[Ca^{2+}]_i$ (as measured using the Ca^{2+} indicator indo-1), and such rises were also strongly inhibited by organic L-type Ca^{2+} channel blockers.[18] Thus in rat type I cells at least, close parallelisms exist between mechanisms for transduction of hypoxic and hypercapnic stimuli.

CONCLUSIONS

In conclusion, all the carotid body chemoreceptor cells studied so far, from adult or neonatal rabbits or rats possess voltage dependent currents. However there are numerous differences in the properties of those currents reported in the literature (see Table 4.1 for a resume).Several possible explanations could account for these differences. First, differences in the stage of development of the cells. The lack of Na^+ currents in rabbit embryos vs. adult rabbit may be an example of that. Secondly, differences occurring between species, as the lack of a transient K^+ current in rat cells, or the different types of K^+ currents modulated by O_2 in rat vs. rabbit may be such an example. Thirdly, differences in the isolation procedures or the conditions or durations of culturing of the cells may be an important factor. This might explain why Na^+ currents are recorded in neonatal rat cells by some authors that keep cells in culture for longer than 48h but not by others that use freshly isolated or short term cultured cells.

Despite these remaining discrepancies and questions, the fundamental scheme for the membrane model for hypoxic transduction, i.e., that hypoxic inhibition of K^+ current leads to depolarization/increased excitability, and thus Ca^{2+} influx through voltage-gated channels leading to transmitter release, appears to the best framework to keep searching for the actual mechanisms involved in hypoxic chemotransduction.

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