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Topical Review

Transduction of Chemostimuli by the Type I Carotid Body Cell

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Introduction

The carotid bodies are the principal arterial chemoreceptors and are located at the bifurcation of each of the common carotid arteries. Each is a highly vascularized organ and senses changes of arterial PO_2, PCO_2 and pH. Arterial hypoxia, hypercapnia or acidosis cause an increase in firing frequency of afferent chemosensory fibers of the carotid sinus nerve (CSN) which in turn influences the integrated output of the central respiratory center. In this way the carotid body can initiate or modify respiratory (and also cardiovascular) reflexes in order to maintain PO_2, PCO_2 and pH levels within physiologically acceptable levels [26]. The carotid body is compact and structurally complex (Fig. 1): the endings of afferent chemosensory fibers are in close, synaptic contact with clusters of type I (or glomus) cells. Type I cells are of neuroectodermal origin and share many anatomical features with adrenal chromaffin cells. They are near spherical (approximately 10 μm in diameter) and contain a variety of neurotransmitter-filled vesicles of which catecholamines, particularly dopamine, have received the greatest attention [27]. Each cluster of type I cells is encapsulated by glial-like type II (sustentacular) cells, and is in close contact with a dense network of fenestrated capillaries (Fig. 1). It is now generally accepted that the type I cell is the chemosensory element of the carotid body, and the numerous publications which demonstrate correlations between hypoxia- or acid-induced release of neurotransmitters (particularly dopamine) and afferent chemoreceptor fiber discharge point to chemostimulus-induced transmitter release from type I cells as being a fundamental step in carotid body chemotransduction [26, 27, 33].

In 1985, two separate reports [30, 60] indicated that type I cells could be dispersed from carotid bodies, maintained in tissue culture and still retain chemoreceptor properties; i.e., hypoxia could stimulate neurotransmitter release. This article reviews recent studies aimed at elucidating the mechanisms by which physiological stimuli (hypoxia, hypercapnia and acidosis) might trigger neurosecretion from isolated type I cells. Given that type I cells are of neuroectodermal origin, it is perhaps not surprising that much research effort has concentrated upon characterizing the electrophysiological properties of isolated type I cells. A brief overview of this work is presented first as background to the research into transduction mechanisms.

Type I Cells are Electrically Excitable

Over the past six years, application of whole-cell and single channel patch clamp techniques by a number of laboratories have shown that isolated type I cells from rabbits and rats possess both inward and outward ionic conductances. In adult rabbit type I cells, voltage-gated inward currents consist of rapidly activating and inactivating, tetrodotoxin-sensitive Na^+ currents, and sustained Ca^{2+} currents (I_{Ca}) [24, 44, 80]. In the rat type-I cell, however, Na^+ channels are either absent [29, 55], or only present at low density [78]. In both rat and rabbit type-I cells I_{Ca} is at least partly due to activation of L-type Ca^{2+}

Key words: Carotid body — Hypoxia — Acidosis — Ion channels — Calcium

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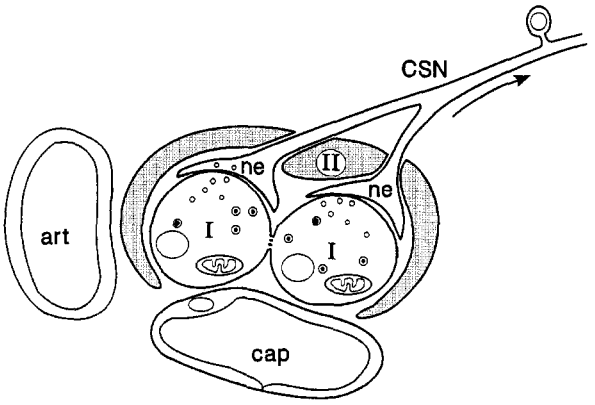

Fig. 1. Schematic diagram of the cellular arrangement of the carotid body (courtesy of Dr. P.C.G. Nye). Note the vesicle-containing type I cells (*I*) which are in close apposition to afferent carotid sinus nerve (*CSN*) endings and encapsulated by type II cells (*II*). The organ also has a rich blood supply (*cap*, capillary; *art*, arteriole). Autonomic innervation is not illustrated. Note that the two type I cells are coupled.

channels [29, 51, 55]. In addition, several studies suggest the presence of other types of voltage-operated Ca^{2+} channels (although as yet there has been no comprehensive pharmacological characterization of I_{Ca} in these cells). In fetal rabbit type I cells, high concentrations of organic Ca^{2+} channel blockers (presumed selective for L-type channels) do not fully block I_{Ca} [38], and in adult rabbit cells, tail current analysis has suggested the possible presence of two populations of Ca^{2+} channels [80]. Interestingly, a recent study in rat type I cells has only identified L-type Ca^{2+} channels using single channel recording techniques, yet the whole-cell Ca^{2+} channel current cannot be fully blocked by a high concentration (10 μM) of nifedipine, leading the authors to suggest that there may be dihydropyridine-sensitive and dihydropyridine-insensitive types of L-type channels in these cells [29].

As in many other excitable cells [71], type I carotid body cells possess a variety of outward K^+ channels, and some of their more important properties have been summarized in the Table. All studies to date have revealed both Ca^{2+}-sensitive (K_{Ca}) and Ca^{2+}-insensitive components of whole-cell K^+ currents. K_{Ca} currents have generally been shown to be of the high conductance (maxi K or BK) variety, as demonstrated via single channel recording and charybdotoxin-sensitivity of whole-cell K_{Ca}, but one report (Table) has also identified an apamin-sensitive (therefore presumably low conductance) K_{Ca} [24]. Although not all groups have fully characterized the subtypes of K^+ channels present in type I cells, these channels have been the focus of most attention to date, since their responses to chemostimuli appear to be of central importance in chemotransduction (*see below*).

Adult rabbit type I cells, perhaps not surprisingly, are capable of generating action potentials [24, 47] but beyond this observation there is no consensus as to their importance: in some instances, action potentials have been shown to occur spontaneously [47], whereas another report suggests that they are evoked only by depolarizing current pulses [24]. In neonatal rat type I cells, action potentials can only be evoked under normal physiological conditions by depolarizing current injection [78].

Chemotransduction of Hypoxic Stimuli

Hypoxia Raises $[Ca^{2+}]_i$ in Type I Cells: A Possible Signal for Neurosecretion

Early studies of dopamine release from the intact carotid body and isolated type I cells have shown that the neurosecretory response to hypoxia requires extracellular Ca^{2+} [28, 30]. It was also reported that hypoxia stimulates $^{45}Ca^{2+}$ influx into cultured type I cells [60]. These findings pointed to stimulus-induced rises in $[Ca^{2+}]_i$ as being central to type I cell chemotransduction. This concept has received much support in recent years. Further studies of catecholamine secretion in response to hypoxia have confirmed a requirement for extracellular calcium [34, 51], and several microfluorimetric studies on isolated type I cells have shown that $[Ca^{2+}]_i$ does indeed rise in hypoxia [7, 8, 13, 34, 43, 73]. The rise in type I cell $[Ca^{2+}]_i$ is graded, showing a relationship with PO_2 similar to that observed for afferent fiber discharge [7, 13]. The hypothesis that a rise of $[Ca^{2+}]_i$ in hypoxia is the signal to neurosecretion is not universally accepted however. Donnelly and Kholwadwala [21] have recently observed that $[Ca^{2+}]_i$ *decreases* in response to hypoxia in acutely isolated rat type I cells and have suggested that an increase in Ca^{2+} binding to an internal site regulates secretion.

The source of the hypoxic rise in type I cell $[Ca^{2+}]_i$ has also been a subject of some controversy. Duchen and Biscoe [7] provided evidence that the rise of $[Ca^{2+}]_i$ resulted primarily from Ca^{2+} release from intracellular stores. They further demonstrated that a similar rise of $[Ca^{2+}]_i$ could be induced by a variety of inhibitors of mitochondrial metabolism (cyanide, rotenone and FCCP; all of which are potent chemostimulants) and proposed that some of the Ca^{2+} released might come from the mitochondrion itself [6–9]. In a further series of studies Duchen and Biscoe [22, 23] elegantly demonstrated a graded depolarization of mitochondrial membrane potential and rise of NAD(P)H with hypoxia (Fig. 2*A*). These events not only correlated with changes in $[Ca^{2+}]_i$ but also demonstrated that mitochondrial metabolism in the type I cell is unusually sensitive to changes in PO_2.

More recent research, however, suggests that the source of Ca^{2+} is other than the mitochondrion (or indeed

Table. K^+ channels in type I carotid body cells

Preparation	Channel type	Conductance, pS ($[K^+]_o$:$[K^+]_i$, mM)	Features	Reference
Adult rabbit	K_{Ca}	207 (130:130)	Ca^{2+} sensitive, v-g	[32, 59, 80]
	K_{Ca}	ND	Ca^{2+} sensitive, apamin sensitive	[24]
	SK	16 (130:130)	Ca^{2+} insensitive, weakly v-g	[32]
	K_{O_2}	42 (130:130)	Ca^{2+} insensitive, v-g, inhibited by 4-AP, cAMP, $\downarrow Po_2$, $\downarrow pH$	[31, 32, 80]
Fetal rabbit	K_{Ca}	ND	Ca^{2+} sensitive, v-g, indirect block by Co^{2+}	[38]
	K_v	ND	Ca^{2+} insensitive, v-g	[38]
	K_{IR}	137 (140:140)	Inwardly rectifying, inhibited by $\downarrow Po_2$	[19]
Neonatal rat	K_{Ca}	190 (120:120)	Ca^{2+} sensitive, v-g, inhibited by charybdotoxin, $\downarrow Po_2$, $\downarrow pH_i$	[53–55, 85]
	K_v	ND	Ca^{2+} insensitive, v-g, inhibited by 4-AP	[54, 56]

Data compiled from the references cited, where either whole-cell or single channel techniques were used. Cells used for studies were kept in short-term culture (4–96 hr). Abbreviations: v-g, voltage-gated (i.e., activated by depolarization); 4-AP, 4-aminopyridine; ND, not determined.

any other internal store). In both rabbit [34, 43] and rat [13] type I cells the hypoxic rise of $[Ca^{2+}]_i$ was completely inhibited in the absence of external calcium. In the rat type I cell the rise of $[Ca^{2+}]_i$ was also greatly attenuated by inorganic Ca^{2+} channel antagonists and dihydropyridines [13]. In intact carotid bodies the increase in both afferent CSN discharge and dopamine release are also inhibited by Ca^{2+}-free media and attenuated by L-type Ca^{2+}-channel antagonists [50, 51, 74, 75]. These data suggest that the hypoxic rise of $[Ca^{2+}]_i$ is mediated, at least in part, by Ca^{2+} influx through voltage-gated Ca^{2+} channels. Hypoxia has also been shown to cause membrane depolarization in fetal rabbit type I cells [19] and to increase the frequency of spontaneous electrical activity in adult rabbit type I cells [43, 47]. Furthermore, in rat type I cells, it has recently been reported [13] that the rise of $[Ca^{2+}]_i$ in hypoxia is not only coincident with membrane depolarization and electrical activity but is also largely dependent upon these events: simultaneous measurements of membrane potential and $[Ca^{2+}]_i$ revealed that anoxia depolarized type I cells and caused a rapid rise of $[Ca^{2+}]_i$, but when cells were voltage-clamped at their resting potentials, anoxia produced a smaller and, importantly, a far slower rise of $[Ca^{2+}]_i$ [13].

Most of the experimental evidence, therefore, currently favors a transduction mechanism for hypoxia which involves modulation of the electrical properties of type I cells to bring about either a membrane depolarization and/or an increase in electrical activity; this promotes Ca^{2+} influx through voltage-gated channels, a rise in $[Ca^{2+}]_i$, and thus neurosecretion.

Hypoxia Inhibits K^+ Channels in Type I Cells. A Possible Mechanism for Hypoxic Chemotransduction

Lopez-Barneo and colleagues first reported in 1988 [44] that hypoxia could inhibit a K^+ current in adult rabbit type I cells, while Na^+ and Ca^{2+} currents were unaffected. This was rapidly followed by similar reports of K^+ channel inhibition by hypoxia from other laboratories working on fetal rabbit and neonatal rat type I cells. More detailed studies regarding the nature of these K^+ channels, however, have shown some discrepancies. In the adult rabbit type I cell hypoxia selectively inhibited a voltage-gated, Ca^{2+}-insensitive K^+ channel, with a single channel conductance of about 40 pS, termed the Ko_2 channel [31, 32] (*see also* the Table). These findings contrast with those using neonatal rat type I cells, where whole-cell recordings have shown that hypoxia selectively inhibits a large conductance charybdotoxin-sensitive Ca^{2+}-activated K^+ current [53, 54]. In fetal rabbit cells, the nature of the O_2-sensitive K^+ channel has not been fully characterized but a single channel conductance of 137 pS has been reported [19]. Interestingly, K^+ currents suppressed by hypoxia have more recently been identified in other O_2 sensing tissues, namely pulmonary artery smooth muscle [61, 89] and airway neuroepithelial cells [88]. As in the carotid body, there is no consensus as to the K^+ channel type which is inhibited by hypoxia, but the basic observation that O_2-sensitive K^+ channels exist in O_2-sensing tissues points to similarities in the sensing mechanism in these tissues; i.e., hypoxia leads to closure of K^+ channels causing cell depolarization which in turn triggers Ca^{2+} influx through voltage-gated Ca^{2+} channels [48, 87].

Modulation of K^+-Channel Activity by Hypoxia

Since the publication of the above-mentioned observations, several groups have investigated the possible mechanism(s) by which hypoxia can inhibit K^+ channel activity. In rabbit type I cells the inhibitory action of hypoxia is retained in single channel recordings from isolated membrane patches indicating that oxygen mod-

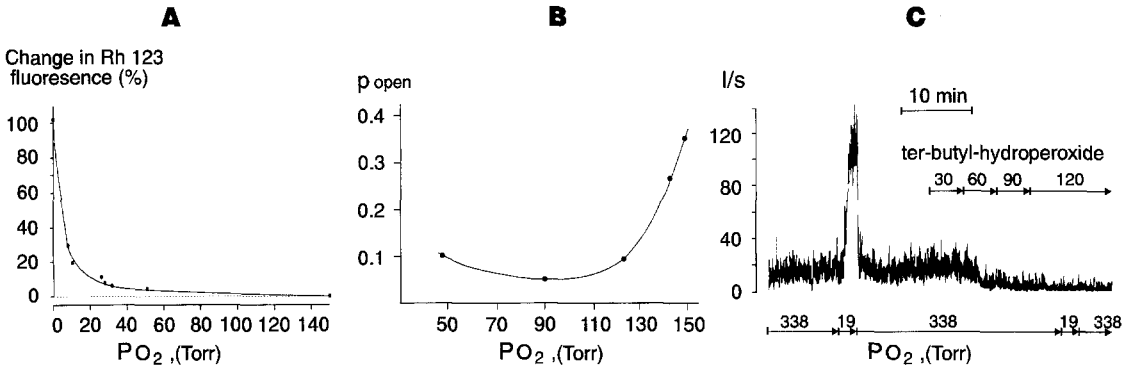

Fig. 2. (*A*) Measurement of rhodamine-1,2,3 fluorescence as a function of PO_2, measured in an isolated type I cell preparation from the adult rabbit. Increasing fluorescence corresponds to depolarization of the mitochondrial membrane potential. (*B*) Single K^+ channel open probability (P_{open}) as a function of PO_2. Data were obtained from a patch of membrane excised from an adult rabbit type I cell. Note that the channel is most sensitive to PO_2 over the range 150–120 Torr. (*C*) Rat CSN discharge measured in vitro. Note that hypoxia excites discharge, but this effect is abolished in the presence of ter-butyl-hydroperoxide. Figures taken from references [1, 23 and 31], with permission.

ulates open channel probability through a membrane-delineated mechanism [31, 32]. Furthermore, channel inhibition in this species is extremely rapid and can be fully reversed by carbon monoxide, strongly suggesting that K^+ channels are closely coupled to a haem-containing protein [46]. However, one criticism of these studies is that the relationship between PO_2 (partial pressure of oxygen) and K^+ channel activity is displaced so that channel inhibition is seen over PO_2 ranges which do not excite the intact carotid body [31, 47] (Fig. 2*B*). Direct regulation of K^+ channel activity by PO_2 alone cannot therefore account for the oxygen-sensing properties of the intact organ. However, there may be an additional pathway through which oxygen can modulate K^+ channel activity. Cyclic AMP has been shown to rise in type I cells under hypoxic conditions [18, 58, 82, 83], and has also been demonstrated to inhibit O_2-sensitive K^+ currents in rabbit type I cells [45]. This dual regulation of K^+ channels (i.e., through the membrane-delineated mechanism and via cAMP) may give rise to a more physiologically relevant relationship between PO_2 and channel activity. This hypothesis remains to be tested, however. Elevated cyclic AMP levels seen in hypoxia may also serve to potentiate transmitter release regardless of channel modulation, since this cyclic nucleotide has recently been shown to potentiate insulin secretion in a Ca^{2+}-independent manner from pancreatic β-cells [3].

An alternative (or perhaps additional) mechanism by which K^+ channels may be directly regulated by O_2 tension comes from Acker and colleagues [1, 17]. These workers suggest that the O_2 sensor is a cytochrome-b which is probably linked to an NADPH-oxidase. Under normoxic conditions this cytochrome-b is proposed to synthesize superoxide (O_2^-) which is then converted to H_2O_2 by superoxide dismutase. This H_2O_2 is then further reduced to H_2O by glutathione peroxidase with consequent oxidation of glutathione (the major cellular pool of mobile thiol groups). Hypoxia inhibits the NAD(P)H oxidase so that H_2O_2 production falls, with a consequent shift in the redox equilibrium of glutathione. Thus, hypoxia increases the reduced form of glutathione, which may inhibit K^+ channel activity (as has been shown for other K^+ channels expressed in *Xenopus* oocytes [72]). A key observation to support this idea is shown in Fig. 2*C*, where provision of an organic peroxide inhibits hypoxic stimulation of the intact carotid body, as measured by chemosensory fiber activity.

Chemotransduction of Acidic and Hypercapnic Stimuli

Changes in Intracellular pH are Central to Acid Chemotransduction

There is considerable evidence that the first stage in the chemotransduction of acidic stimuli involves the acidification of type I cell intracellular pH (pH_i). The first indication of this was the observation that membrane permeant carbonic anhydrase inhibitors (e.g., acetazolamide) slowed and attenuated the normally rapid increase CSN discharge in response to a respiratory acidosis in vivo [36]. These effects have recently been confirmed in saline perfused carotid bodies in vitro [39, 40, 68]. Since carbonic anhydrase accelerates the hydration of CO_2, this suggests that production of H^+_i and HCO_{3i}^- is an important step in the transduction of a hypercapnic acidosis. The presence of carbonic anhydrase within type I cells has been confirmed histologically [49, 66], and by measuring the effects of acetazolamide upon CO_2-induced changes in pH_i [16].

A further indication as to the role of pH_i is that the carotid body can be excited by many different acidic

stimuli including hypercapnic acidosis (increase in P_{CO_2} and decrease pH_o), isocapnic acidosis (decrease in pH_o at constant P_{CO_2}), isohydric hypercapnia (increase in P_{CO_2} at constant pH_o), weak acids and brief exposure to NH_4^+ [35, 41, 68]. The only common feature of these stimuli is that they all decrease pH_i; no other single factor (i.e., CO_2 HCO_3^- or pH_o) changes consistently with all the above stimuli [11, 14, 15, 41]. In addition, only those stimuli which induce a decrease in steady-state pH_i (hypercapnic and isocapnic acidosis) provoke a sustained increase in chemoreceptor activity. Isohydric hypercapnia, which causes only a transient fall in pH_i [15], provokes only a temporary increase in chemoreceptor activity [35].

pH_i Regulation in Type I Cells is Tightly Controlled

Given the proposed role of pH_i in acid chemotransduction, pH_i regulation in type I cells must be considered to play an important part in the transduction process. In physiological salines containing HCO_3^- and CO_2 the resting pH_i of rat type I cells is approximately 7.2 [16, 84] and is closely regulated. Acid extrusion from the type I cell is achieved through the Na^+/H^+-exchanger [16, 84] and a Na^+-HCO_3^--dependent process [16]. Na^+/H^+ exchange mediates an electroneutral (1:1) exchange of internal protons for external Na^+ [4], and is sensitive to changes in both pH_i and pH_o. A fall of pH_i markedly enhances Na^+/H^+ exchange [16], whereas a fall of pH_o inhibits it [4, 81]. The Na^+-HCO_3^--dependent acid extrusion mechanism in type I cells is thought to mediate a co-influx of Na^+ and HCO_3^- into the cell (HCO_3^- influx is equivalent to H^+ efflux) [16]. Recent research indicates that this transporter is also Cl^- dependent [64] and may therefore be similar to the Na^+-dependent HCO_3^-/Cl^- exchanger, or four ion carrier [70].

Three potential acid-influx mechanisms have also been identified in type I cells. These are a Na^+-independent Cl/HCO_3^--exchanger [16], an HCO_3^--permeable anion channel [77, 79] and a K^+/H^+ exchange [84]. The Na^+-independent $Cl^-HCO_3^-$ exchange mediates net exchange of external Cl^- for internal HCO_3^- (i.e., acid-influx). In other tissues, Cl^-/HCO_3^- exchange activity is increased by intracellular alkalosis [2]. The anion channel was first identified in inside-out membrane patches from cultured rat type I cells; it has a large unitary conductance (296 pS) and a HCO_3^-/Cl^- permeability ratio of 0.7 [77]. Assuming a resting membrane potential of −50 mV, this channel would permit a net efflux of HCO_3^- and could therefore be a major route for acid-equivalent influx. Its contribution to acid-influx and its role in pH_i regulation, however, remain to be quantified. An electroneutral K^+/H^+ exchanger has been reported in the adult rat type I cell [84] and is thought to mediate a net exchange of intracellular K^+ for extracellular H^+. In the neonatal rat type I cell, however, endogenous K^+/H^+ exchange seems to be absent [65] but can be introduced *in situ* by inadvertent contamination with the K^+/H^+-ionophore nigericin [65] (nigericin is used extensively for *in situ* calibration of pH-sensitive fluoroprobes and can contaminate cell perfusion systems).

In view of the ability of the type I cell to regulate pH_i, one might question whether changes in pH_i can have any role in acid chemotransduction. However, while the type I cell is able to correct for internal acid/base challenges, the steady-state level for pH_i is extremely sensitive to changes in pH_o [15, 37, 84]. This is true regardless of whether pH_o is changed by varying P_{CO_2} (e.g., Fig. 3*A*) or by varying $[HCO_3^-]_o$ [15] or even in a nominally CO_2/HCO_3^--free medium [84]. Indeed, steady-state pH_i changes by some 65 to 82% of any change in pH_o [15, 84]. This unusual sensitivity of pH_i to changes in pH_o suggests a functional specialization of pH_i regulation in type I cells.

Acid Chemoreception Involves a Rise in Type I Cell $[Ca^{2+}]_i$

While a fall in pH_i in the type I cell is probably the initial event in acid chemoreception, there is also evidence that $[Ca^{2+}]_i$ plays an important role: acid-induced neurosecretion from type I cells is dependent upon $Ca^{2+}{}_o$ and an acid-induced rise in type I cell $[Ca^{2+}]_i$ may serve as the signal to neurosecretion [69]. The effects of acidity upon type I cell $[Ca^{2+}]_i$, however, are controversial. Biscoe and Duchen [7] found that lowering pH_o had no effect upon $[Ca^{2+}]_i$ under normoxic conditions, although it substantially enhanced the rise of $[Ca^{2+}]_i$ induced by hypoxia. More recently, Buckler and Vaughan-Jones [11] have reported that hypercapnic acidosis (e.g., Fig. 3*B*), isocapnic acidosis and isohydric hypercapnia all raise $[Ca^{2+}]_i$ in neonatal rat type I cells. All of these maneuvers also stimulate chemoreceptor discharge [35] and neurosecretion [67, 68]. Furthermore, the averaged $[Ca^{2+}]_i$ response to each stimulus had a characteristic pattern which resembled the different effects these same stimuli have upon chemoreceptor discharge. Briefly, both the rise of $[Ca^{2+}]_i$ and the increase in CSN discharge displayed the following properties: (i) the initial response to hypercapnia is more rapid than that to an isocapnic acidosis; (ii) the response to both a hypercapnic acidosis and an isohydric hypercapnia display marked adaptation and (iii) the response to an isohydric hypercapnia is mostly transient with little or no sustained increase in $[Ca^{2+}]_i$ or CSN discharge. The observed rise of $[Ca^{2+}]_i$ in response to acidic stimuli is therefore consistent with a central role for $[Ca^{2+}]_i$ in acid chemotransduction. Thus, the transduction pathway for acidic stimuli

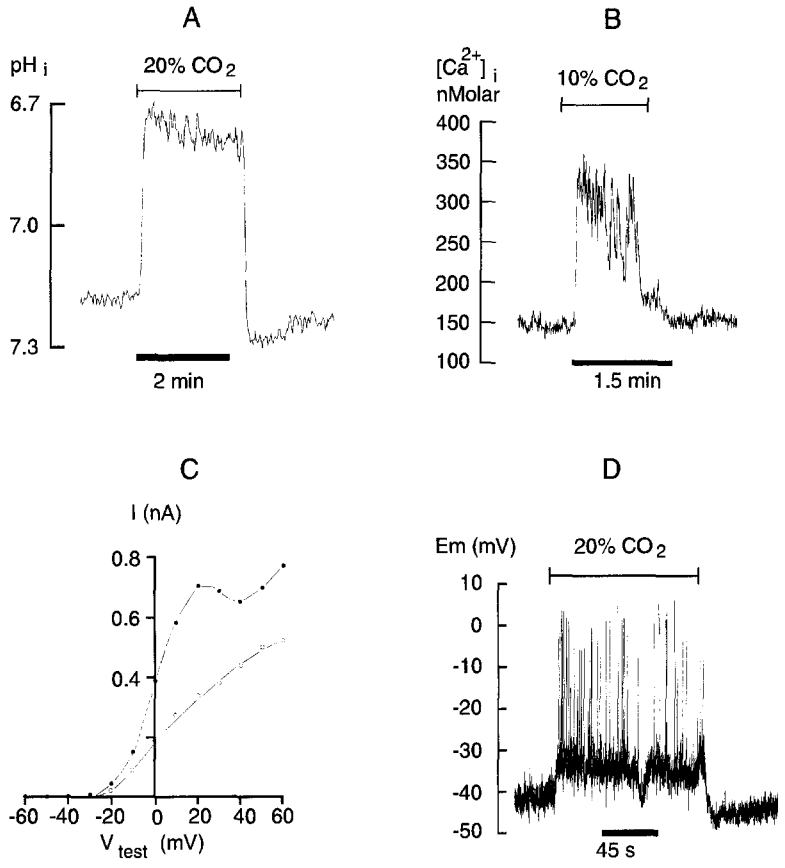

Fig. 3. (*A*) Response of pH_i in a neonatal rat type I cell to an increase of CO_2 from 5 to 20%, measured with a pH-sensitive fluoroprobe. (*B*) Response of intracellular $[Ca^{2+}]$ to an increase in CO_2 of the perfusate (from 5 to 10%) as indicated. (*C*) K^+ current-voltage relationship obtained from an isolated neonatal rat type I cell at pH_o 7.4 (filled circles) and 7.0 (open circles). (*D*) Recording of membrane potential from a type I cell during exposure to a solution of 20% CO_2 (20% $CO_2 \approx 150$ Torr). Note a depolarization with superimposition of spiking activity in the hypercapnic condition. Figures taken from references [11, 12 and 52], with permission.

probably involves a fall in pH_i which induces a rise in $[Ca^{2+}]_i$, and this in turn triggers neurosecretion.

Mechanisms of Ca^{2+} Signaling in Response to Acid Stimuli

The observation that the secretory response to acidic stimuli is dependent upon the presence of $Ca^{2+}{}_o$ [69] suggests that Ca^{2+} influx plays an important role in mediating the rise of $[Ca^{2+}]_i$. Indeed, in neonatal rat type I cells, the rise in $[Ca^{2+}]_i$ induced by hypercapnic acidosis is abolished in $Ca^{2+}{}_o$-free media, and substantially inhibited by 2 mM Ni^{2+} [11, 12]. A hypercapnic acidosis also increases Mn^{2+} influx into type I cells [12], which suggests that acidosis increases cell membrane Ca^{2+} permeability. Together, these data argue convincingly that the rise of $[Ca^{2+}]_i$ during acidic stimulation of the type I cell results from Ca^{2+} entry from the external medium. Two contrasting mechanisms linking a fall in pH_i to an increase in Ca^{2+} influx and a rise in $[Ca^{2+}]_i$ have been proposed, the Na^+/Ca^{2+}-exchange hypothesis and the membrane potential hypothesis.

The Na^+/Ca^{2+}-exchange hypothesis [33, 69] proposes that the following steps lead to the rise of $[Ca^{2+}]_i$: (i) acid stimuli induce a fall in pH_i which activates Na^+-dependent acid extrusion mechanisms (i.e., Na^+/H^+-exchange and Na^+-HCO_3^--co-influx); (ii) the resultant increase in Na^+ influx raises $[Na^+]_i$, and (iii) the rise in $[Na^+]_i$ promotes reverse mode Na^+/Ca^{2+} exchange which brings Ca^{2+} into the cell (*see* Fig. 4*B*). In the rabbit type I cell, several lines of evidence point to this mechanism [69]. Most notably, acid-induced neurosecretion is inhibited by the absence of extracellular Na^+ or Ca^{2+}, is partly inhibited by EIPA (a Na^+/H^+-exchange inhibitor), but is not inhibited by nisoldipine (an L-type Ca^{2+}-channel antagonist). In support of this hypothesis, activation of Na^+/H^+ exchange by a fall in pH_i has been confirmed in rat type I cells [16, 84], but the net effect of a fall in both pH_i and pH_o (as occurs with a respiratory or metabolic acidosis) upon Na^+/H^+ exchange has not been determined (NB Na^+/H^+ exchange is inhibited by a fall of pH_o). There is also some evidence for the existence of an Na^+/Ca^{2+}-exchanger in the rabbit type I cell. Biscoe et al. [9] noted that the removal of extracellular Na^+ caused a rise in basal $[Ca^{2+}]_i$ and slowed the rate of $[Ca^{2+}]_i$ recovery following depolarization in high K^+.

Much of the data upon which the Na^+/Ca^{2+}-exchange model for acid chemotransduction is based [69], however, is open to alternative interpretation. For example, the inhibition of acid-induced neurosecretion in $Na^+{}_o$-free media could result from membrane hyperpolarization [12], and the effects of EIPA could result from

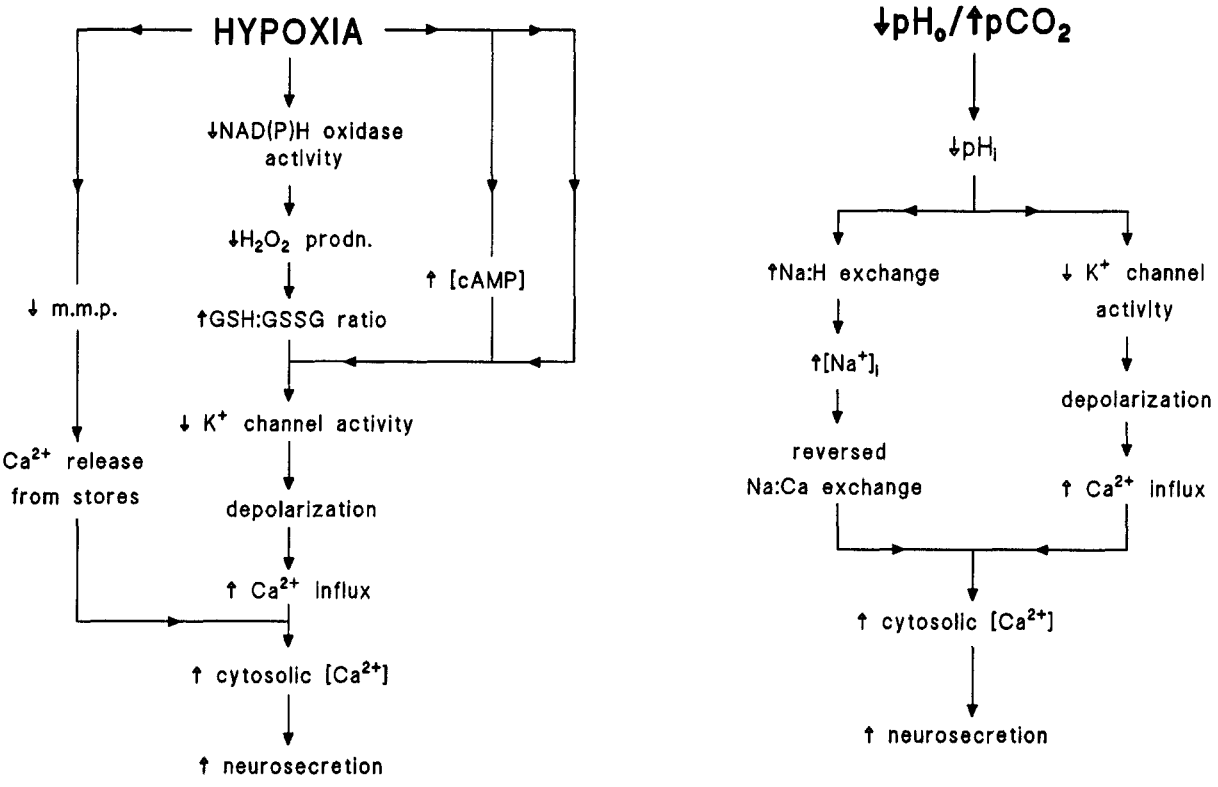

Fig. 4. Flow diagrams summarizing the postulated mechanisms for transduction of hypoxic (left) and acidic (right) stimuli in type I carotid body cells. (*m.m.p.*), mitochondrial membrane potential.

various known interactions between this drug and ion channels, receptors, second messenger systems or other targets [42]. More importantly, much of the data upon which the Na^+/Ca^{2+}-exchange hypothesis is based was obtained using the protonophore 2,4-dinitrophenol (DNP; a very powerful chemostimulant), on the assumption that this causes a very large fall of pH_i [69]. Direct measurements in rat type I cells, however, show that DNP induces only a small fall in pH_i (>0.1 pH unit), but a very large rise in $[Ca^{2+}]_i$ [10]. This suggests that the potent stimulatory effects of DNP upon $[Ca^{2+}]_i$, neurosecretion and CSN discharge, have little to do with its limited effects upon pH_i.

The basic concept of the membrane potential hypothesis is that acidic stimuli inhibit K^+ channels which leads to membrane depolarization and Ca^{2+} entry through voltage-gated Ca^{2+} channels [52, 55, 76]. K^+ channels in type I cells are indeed inhibited by acidic stimuli [47, 52, 56, 76] (*see* Fig. 3*C*). In the rat type I cell, this effect appears to be principally mediated by a fall of pH_i [55, 76] acting upon the large conductance Ca^{2+}-activated K^+ channel [52, 55]. Further to this, most type I cells depolarize in response to lowering pH_o to 5.5 [37]. Two recent lines of evidence now directly support the membrane potential hypothesis for acid transduction. First, the Ca^{2+} response to hypercapnic acidosis is partially inhibited by the L-type Ca^{2+}-channel antagonists, nicardipine and D600 [12, 14]. Second, simultaneous recordings of membrane potential and $[Ca^{2+}]_i$ have shown that the rise of $[Ca^{2+}]_i$ induced by hypercapnia is accompanied by membrane depolarization (or a receptor potential, *see e.g.*, Fig. 3*D*) and electrical activity and that preventing these electrical responses by voltage-clamping eliminates the $[Ca^{2+}]_i$ response to hypercapnia [12].

The ion channel/s responsible for the acid-induced receptor potential have yet to be formally identified. Preliminary data obtained using voltage ramps over the range −90 to −30 mV shows that acidosis induces a decrease in resting membrane conductance, and that the acid-sensitive current has a reversal potential of around −75 mV [12]. These data are consistent with the inhibition of a K^+ conductance. Since acidosis is known to inhibit the large conductance K_{Ca} channel in rat type I cells [52, 55] it is tempting to speculate that it is this channel that is involved in generating the receptor potential and increasing electrical excitability in response to acid stimuli.

Neurotransmission and Neuromodulation in Chemoreception

The studies described above have been based on the central dogma that chemotransduction involves transmit-

ter release from type I cells following a rise of $[Ca^{2+}]_i$. However, there is no definitive account of where and how the transmitters act once released. There is a general assumption that transmitters can act at the nerve endings to modify carotid sinus nerve activity, but the picture is certainly not this simple. There is much good evidence that transmitters can also act on the type I cells from which they are released (i.e., on presynaptic or autoreceptors) [25] and this has more recently been investigated using isolated type I cell preparations. In isolated rabbit cells dopamine has been shown to partially inhibit I_{Ca}, indicating that this transmitter regulates its own release by feedback inhibition [5], and in isolated neonatal rat cells activation of nicotinic acetylcholine receptors leads to depolarization and rises in $[Ca^{2+}]_i$, indicative of a positive feedback mechanism in this species [57, 86]. Further to this, it should be noted that afferent nerve endings themselves contain neurotransmitters such as substance P and application of a selective NK-1 receptor antagonist (CP-96,345) has recently been shown to selectively inhibit sensory discharge elicited by hypoxia, indicating an important role for this peptide in chemotransduction [62]. Afferent nerve endings also appear to generate nitric oxide which exerts a tonic inhibitory effect on the intact organ [63].

To date, studies concerning the actions of neurotransmitters on isolated type I cells are in their infancy as attention has been focused on the actions of physiological stimuli (hypoxia, acidity) on type I cells. However, detailed examinations of the actions on type I cells of the wide variety of neurotransmitters present in the carotid body will be required to fully describe the responses of type I cells to chemostimuli in vivo. In this respect, it is noteworthy that electrochemical techniques have been recently applied to the intact carotid body to demonstrate Ca^{2+}-dependent dopamine release in response to hypoxia which correlates well with increases in afferent CSN activity [20].

Summary

The postulated mechanisms for hypoxic and acidic chemotransduction by type I cells that we have described here are summarized in the diagrams of Fig. 4. Most if not all of these require more complete evaluation and, as we have described, there are obvious points of contention that need to be resolved. Nevertheless, it is apparent that studies of isolated type I cell preparations carried out over the last six years have provided significant advancements in our understanding of chemotransduction in the type I cell. Only when the functioning of these cells has been fully described can we hope to understand the mechanisms underlying the responses of the intact organ to chemostimuli.

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