Carotid body chemoreception: The importance of CO_2-HCO_3^- and carbonic anhydrase. (Review)

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The current hypotheses of carotid body (CB) chemoreception regard the glomus cells as the initial site of stimulus transduction. The consensus is that the transduction of chemical stimulus is coupled with the release of transmitter(s) from the glomus cells, which in turn generates action potentials in the afferent nerve terminals. Carbonic anhydrase (CA) is present in the glomus cells of the CB. Inhibition of CA activity in the CB in situ reduces the carotid chemosensory responses to CO_2 and to O_2, suggesting a common mechanism of chemosensing for both stimuli. However, CA inhibitors also block the red blood cell enzyme. Thus, the CO_2 hydration reaction does not come to completion within the transit time of the blood from the lung to the CB. A steady-state reaction is not reached until later and so the PCO_2 and pH levels in arterial blood samples are not the same as those sensed by the CB. Experiments in vitro *using cat CB perfused and superfused with cell-free solutions, which had been pre-equilibrated with respiratory gases, strongly support the proposition that the CA activity in CB cells is essential for the speed and amplitude of the initial response to CO_2 and for its subsequent adaptation. The immediate response to hypoxia also is delayed, but the late steady-state was less dependent on CA activity. In the nominal absence of CO_2-HCO_3^- from the perfusate, hypoxic chemoreception persisted and its magnitude is not affected by CA inhibition, except for a delay which may be due to the initial alkaline pH of the glomus cells. Recent experiments performed in isolated glomus cells and in the whole CB show that hypoxia does not modify significantly the intracellular pH. By its simple catalytic function, CA can speed up the approach of the CO_2 hydration reaction to equilibrium. However, CA may also contribute in the steady-state to the regulation of pHi by providing a continuous supply of H^+ and HCO_3^-. Furthermore, CA performs a facilitatory role in the physiological chemosensory responses to CO_2 and O_2 in the presence of extracellular CO_2-HCO_3^-. This role is likely to be related to the ion exchanger function and then to pH_i regulation in the chemoreceptor cells.*

INTRODUCTION

The carotid body (CB) is located in the carotid bifurcation and is innervated by a branch of the glossopharyngeal nerve: the carotid (sinus) nerve (12). The carotid chemoreceptors sense the partial pressures of respiratory gases and the $[H^+]$ in the arterial blood and initiate the appropriate ventilatory and cardiovascular reflex adjustments. It is well known that hypoxia, hypercapnia and acidosis increase the firing rate of the chemosensory fibers of the carotid nerve, while hyperoxia, hypocapnia and alkalosis markedly decrease it (12, 14). It is also known that carotid chemosensory discharges show strong cooperative CO_2-O_2 stimulus-response interaction (12, 33).

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According to prevailing ideas (12, 14), the glomus (Type I) cells act as the sensors of chemical changes in their environment. Thus, they are the initial site of transduction which causes them to release transmitter(s) which in turn initiate action potentials in the afferent nerve terminals apposed to them (12, 14). The transduction of chemical stimuli in the glomus cells appears to be mediated by second messengers (*e.g.*, Ca^{2+}, cAMP), which trigger the release of transmitter(s). However, the precise mechanisms of chemotransduction are debated and various hypotheses have been advanced (see for review 12 and 14).

CARBONIC ANHYDRASE IN THE CAROTID BODY

In aqueous solution, CO_2 reacts simultaneously in two ways (10,40):

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad \text{(eq 1)}$$

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \quad \text{(eq 2)}$$

The hydration reaction of CO_2 (eq 1) predominates in physiological conditions at 37° C and pH 7.4, while the other reaction (eq 2) is only prominent at above pH 9.0. In general, the hydration of CO_2 to H_2CO_3 in biological systems is catalyzed by the enzyme carbonic anhydrase (CA) (10). Four CA isoenzymes, designated CA-I (or B), CA-II (or C), CA-III (or M) and a membrane bound form (CA-IV), have been described in detail. Each is a single chain peptide of 259 or 260 residues, containing a single Zn atom which is essential for its catalytic activity (10).

The dissociation of carbonic acid into HCO_3^- and H^+ occurs instantaneously in comparison with the CO_2 hydration reaction which is the rate limiting step. At 38° C and pH 7.4, the rate constant of the uncatalyzed CO_2 hydration is 3.5 x 10^{-2} s^{-1} (41). The presence of CA increases the speed of the CO_2 hydration reaction by approximately 2,000 to 13,000-fold, depending on the activity of the enzyme (15, 41, 52). Foster *et al.* (15) reported values for the catalytic rate constant (pseudo-first order) of CO_2 hydration (at pH 7.4 and 37° C) measured in red cells, ranging between 1995 s^{-1} in the cat to 571 s^{-1} in the horse. Because of its simple catalytic function, CA speeds up the

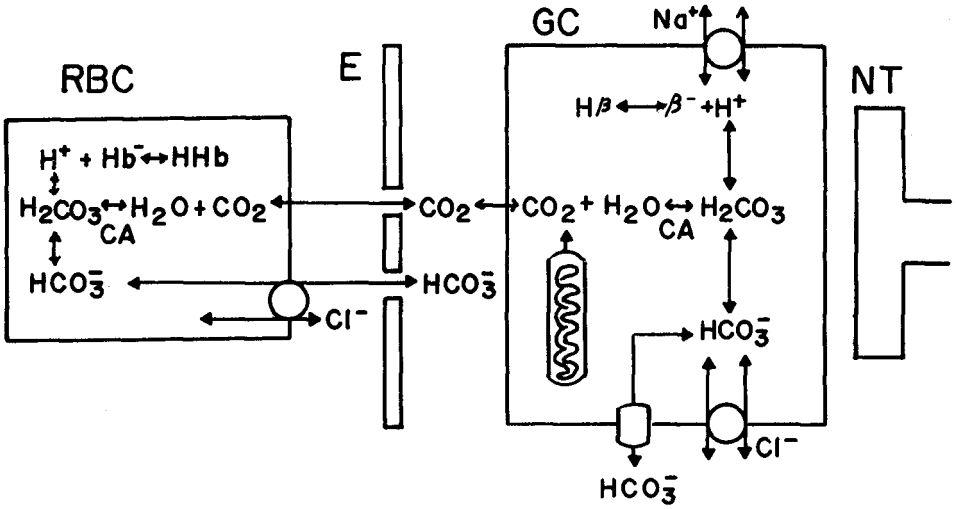

Fig 1: Schematic diagram of the CO_2 pathway in the carotid body. RBC, red blood cell; E, endothelial cells; GC, glomus cell; NT, nerve terminal. CO_2 freely moves through the different cellular compartments depending on its partial pressure. The presence of CA speed up CO_2 hydration to equilibrium in RBC and GC, and also contributes to pH_i regulation in the GC. In steady-state, ion fluxes are affected by the action of CA, by providing a continuous supply of H^+ and HCO_3^-. $[H^+]_i$ in GC depends on the balance of acid extrusion (H^+–Na^+ exchanger), buffer capacity of the cell (β–, anionic protein groups) and HCO_3^- extrusion (anion channel and Cl^-–HCO_3^- exchanger). At this time, we do not know if CA is present in E, NT or in extracellular space.

approach of the CO_2 hydration reaction to equilibrium. But, CA also contributes to the regulation of intracellular pH (pH_i) in the steady-state, in which the ion fluxes are affected by the action of CA by providing a continuous supply of H^+ and HCO_3^- (Fig 1). The CO_2-HCO_3^- pair in the absence of CA is a poor unstirred-layer buffer because the slow spontaneous hydration reaction limits the rate at which H^+ can be buffered (2). In many cells, CA mediates such different physiological processes as reversible catalysis of CO_2 hydration, HCO_3^- dehydration, facilitation of CO_2 diffusion and H^+ production (10, 41, 52).

The enzyme CA is present in the glomus cells of the CB (37, 43, 48, 49). Ridderstrale and Hanson (48) and Rigual *et al.* (49), using histochemical techniques, reported that apparently only the glomus cells contain CA. Sustentacular (Type II) cells and the endothelia of the fenestrated capillaries or sinusoids did not stain and only a very few axons in the carotid nerve were stained. Consequently, we can assume that the effects of CA occur in the cytoplasm of the glomus cells, but we do not know the precise distribution or the isoenzyme type of the CA present in the glomus cells.

Sulfonamides like acetazolamide and benzolamide have been used *in situ* to assess the physiological function of the enzyme in the cat CB chemosensory process (1, 4, 20, 21, 36). Those drugs are commonly used to inhibit CA because of their specificity and strong inhibitory potency at concentrations from 10^{-6} to 10^{-9} M (10, 41). Torrance and his colleagues (4, 20, 21) found in the cat that the fast overshoot of the carotid chemosensory response to a sudden increase of arterial blood PCO_2 and the subsequent adaptation were reduced by acetazolamide, indicating that an H^+ stimulus is responsible for the response. This result indicates that molecular CO_2 is inert in the transient response, and that the carotid response to CO_2 is mediated by CA. Hayes *et al.* (21) observed that acetazolamide diminishes the magnitude of the chemosensory responses of the cat CB *in vivo*, to both hypercapnic and hypoxic stimuli. They found that the responses to all combinations of end-tidal PO_2 and PCO_2 were diminished by acetazolamide. Other workers, also using the cat CB confirmed the effect of CA inhibitors on CO_2 and O_2 chemoreception (1, 36).

The above observations provided some support for Torrance's hypothesis (20, 61, 62) that a common mechanism of acidification mediated carotid chemoreception to O_2 and to CO_2. He suggested that the carotid nerve endings are sensitive only to acidity, and the extracellular pH of the nerve endings environment is held more stable than that of the blood by an active mechanism which becomes less effective in hypoxia (61). Subsequently, Torrance and colleagues (20) reported that benzolamide, a non-permeating CA inhibitor, is less effective in slowing the chemosensory response to high CO_2 than is acetazolamide. Since acetazolamide does permeate through the cell membrane, the acid receptor theory had to be altered from considering an extracellular receptor to considering an intracellular one instead (see ref 20).

The acid hypothesis of Torrance is attractive because it gives a simple explanation for CO_2-O_2 interaction and for adaptation to CO_2. However, the function of CA in the CB cannot be accurately measured in studies performed on the whole animal because CA inhibitors also block the enzyme in the red blood cells (Fig 1). The CO_2 hydration reaction does not come to completion during the transit time of the blood from the lung to the CB capillaries (4, 32, 41, 52, 60). The steady-state reaction is not reached until later, and the PCO_2, pH and PO_2 levels measured in a sample of arterial blood are not the same as those sensed by the chemoreceptor cells. The disequilibrium produces an apparent arterial-alveolar PCO_2 gradient (9, 60). Thus, the levels of PCO_2 and H^+ stimuli are overestimated in an arterial blood sample, but are underestimated by sampling the end tidal PCO_2 (see for discussion, 21, 32 and 60). This means that the actual stimuli levels at the CB are unknown.

To avoid the problem of disequilibrium in the red blood cell CO_2-HCO_3^- system after CA inhibition, it is necessary to use cell-free perfusion media, previously equilibrated with respiratory gases for studying stimulus-response relationships in the CB.

CAROTID CHEMOSENSORY RESPONSES IN THE ABSENCE OF CO_2-HCO_3^-

Recently, Iturriaga *et al.* (30) have developed a suitable perfused and superfused cat CB preparation which permits the recording of carotid chemosensory discharges for several hours. The cat CB perfused and superfused with Tyrode solution free of CO_2-HCO_3^- (HEPES buffer at pH 7.4), responded very quickly to sudden changes of CO_2-H^+ in the perfusate. Perfusion with Tyrode solutions equilibrated at PCO_2 of 38–110 torr resulted in linear increases of the chemosensory frequency of discharge. The half-time of the chemosensory response to acid hypercapnia (a change of PCO_2 from 0 to 60 torr, pH 7.4 to 7.2) including the transit time through the tubing system was between 6-10 s and the response always showed adaptation (29, 30). Administration of nicotine and cyanide increased the frequency of chemosensory discharge in a dose-related manner. In this and other studies we found that hypoxic response does occur in the nominal absence of CO_2-HCO_3^- from the perfusate (25, 29, 30, 34, 35).

EFFECTS OF CA INHIBITION ON THE CAROTID CHEMOSENSORY RESPONSES *IN VITRO* IN THE ABSENCE OF CO_2-HCO_3^-

Iturriaga *et al.* (26, 29, 34) performed experiments on cat CB preparation perfused and superfused with Tyrode solution *in vitro* to study the effects of CA inhibition on the chemosensory responses to natural and pharmacological stimuli. In these experiments, we used a CO_2-free non-bicarbonate buffer solution (HEPES buffer at pH 7.4), as the basic medium for perfusion and superfusion in order to be able to change CO_2-H^+ stimulus from low to high values. We perfused the cat CB with Tyrode containing the permeating CA inhibitor, methazolamide, in a dose large enough (42.5 μM) to inhibit 99.99% of the CA activity (see ref. 41). The baseline chemosensory activity was not significantly reduced by methazolamide application in the absence of CO_2-HCO_3^- (26, 29, 34). The speed and magnitude of the chemosensory responses to nicotine were also not affected (29, 34). Inhibition of CA activity in the CB eliminated, or delayed and reduced, the chemosensory responses to transient high CO_2 stimuli (PCO_2 38–110 torr). Methazolamide blocked the initial overshoot but did not significantly diminish the late response (1–3 min) to prolonged acid hypercapnia (PCO_2 of 60 torr, pH of 7.2). During methazolamide application, the steady-state responses to hypoxia and to perfusate flow interruption were delayed but were not decreased in height. The characteristic exponential relationship in steady-state between the frequency of chemosensory discharges and the perfusate PO_2 between 10 and 450 torr was not affected during administration of methazolamide. However, the speed of the response to hypoxia was significantly attenuated during CA inhibition (26, 29), and this was presumed to be due to an increase in the intracellular pH (pH_i), as was reported in isolated glomus cells in the absence of CO_2-HCO_3^- buffer by Buckler *et al.* (6). They found that acetazolamide increased the pH_i of rat glomus cells and reduced the speed of the pH_i changes caused by 5% CO_2. However, they did not detect any overshoot of the pH_i change caused by CO_2 application even with unpoisoned CA (6). The slow monotonic acidification produced by high CO_2–H^+ in isolated glomus cells (6, 7, 64) contrasts with the fast overshoot of the response produced in chemosensory fibers by CO_2 (4, 20, 25, 29).

CAROTID CHEMOSENSORY RESPONSES IN THE PRESENCE OF CO_2–HCO_3^-

The presence of CO_2–HCO_3^- in the perfusate and superfusate media while keeping pH constant at 7.4, raised the basal chemosensory discharge of the cat CB and augmented the speed and the maximal magnitude of the responses to hypoxia, to perfusate flow interruption and to cyanide, but it did not change the response to nicotine (25, 35). These augmenting effects of CO_2–HCO_3^- occurred even though the pH of the perfusate and superfusate media was the same with and without CO_2–HCO_3^- (25, 35).

The presence of CO_2–HCO_3^- raised the baseline chemosensory activity, and this was

presumed to be due to an increased intracellular acidity. A rise in intracellular PCO_2 may contribute to cell acidification not only because of the production of H^+ mediated by CA action, but also because of the extrusion of HCO_3^- through Cl^-–HCO_3^- exchanger mechanisms and anion channels (Fig 1). The glomus cells do possess a Cl^-–HCO_3^- exchanger (6) as do many other cells (5, 40, 51). The observation that the blockade of the Cl^-–HCO_3^- exchanger by eliminating external Cl^- made rat glomus cells alkaline in the presence of CO_2–HCO_3^- (6, 64) supports the idea that HCO_3^-–dependent acidification is important in glomus cells. There are chloride channels present in glomus cells which have a large conductance and a selectivity for HCO_3^- (53). They also may participate in the process of HCO_3^- extrusion (6).

Iturriaga and Lahiri (27) tested for the participation of chloride channels in the chemosensory responses to low O_2 and high CO_2 of our perfused and superfused cat CB preparation by the application of 9-anthracene carboxylic acid (2 mM), an anion channel blocker. It reduced the baseline discharges and delayed the onset of the responses to hypoxia and hypercapnia, but it did not diminish the steady-state responses. Reduction of external [Cl^-] from 110 to 10 mM by replacing it with gluconate also decreased the baseline discharge and delayed the response to low O_2 (27). However, these effects of Cl^- could in part be attributed to changes in the membrane potential of glomus cells, because the Cl^- conductance does contribute to the resting potential of the glomus cells (44).

Since the response to stimuli of other sensory receptors depends on the factors that determine their background discharge, it is possible that the presence of CO_2–HCO_3^- in the perfusate medium might increase the resting chemoreceptor discharge and an applied stimulus might have a more potent effect in the presence of CO_2–HCO_3^-. However, we found that CO_2–HCO_3^- potentiates the carotid chemosensory response to hypoxia and cyanide, but does not alter the response to nicotine (25, 35). On the other hand, Zapata and Eyzaguirre (65) found that hypercapnia produced by a change of the inhaled gas from air to 5% CO_2 in 20% O_2 increased the frequency of chemosensory discharge but failed to produce a slow receptor potential or only produced a small depolarization. Hypercapnia, as compared with hypoxia or cyanide, was a poor stimulus for eliciting a mass receptor potential. Later, Eyzaguirre *et al.* (13) reported that the most common membrane response to high CO_2 of the glomus cells was depolarization (69%), but they also observed hyperpolarization in some cases (29%). Although it is not possible to rule out a small effect of CO_2 on the resting receptor potential in other sensory receptors, such as the cat muscle spindle, the application of CO_2 suppressed the generation of action potentials, presumably by reducing the pH_i of the nerve terminal (16).

EFFECTS OF A CA INHIBITOR IN THE PRESENCE OF CO_2–HCO_3^-

Recently, Iturriaga *et al.* (26, 28) studied the effects of the CA inhibitor, methazolamide (130 μM), on the chemosensory responses of the perfused and superfused cat CB *in vitro* preparation to hypoxia, hypercapnia, nicotine and cyanide in the presence of CO_2–HCO_3^- in the perfusate. The results showed that methazolamide decreased the baseline chemosensory activity, and it eliminated the initial peak and diminished the late response to acidic hypercapnia (Fig 2). The initial response to hypoxia was also delayed, but the late response was less affected (Fig 3).

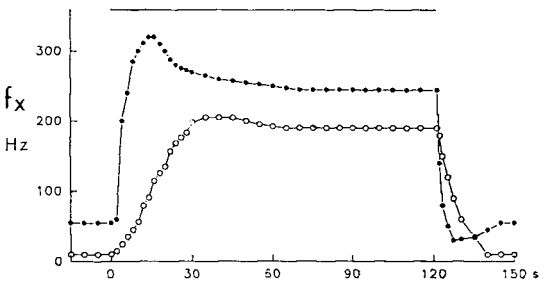

Fig 2: Effects of CA inhibition on the chemosensory response of the cat CB *in vitro* to acid hypercapnia (PCO_2 = 55 torr, pH = 7.15) following normocapnic perfusate (PO_2 = 120 torr, PCO_2 = 34 torr, pH = 7.40). Filled circles, control response; open circles, during methazolamide application (130 μM). f_x, frequency of carotid chemosensory discharges. Bar at the top, duration of hypercapnic perfusion.

Biol Res 26: 319-329 (1993)

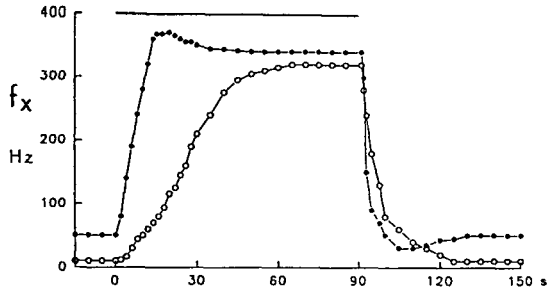

Fig 3: Effects of CA inhibition on the chemosensory response of the cat CB *in vitro* to hypoxia (PO_2 = 30 torr, PCO_2 = 35 torr, pH = 7.40) following normoxic perfusate (PO_2 = 120 torr, PCO_2 = 30 torr, pH = 7.40). Filled circles, control response; open circles, response during methazolamide (130 μM) application. f_x, frequency of carotid chemosensory discharges. Bar at the top, duration of hypoxic perfusion.

Methazolamide also reduced the response to cyanide, but not to nicotine. Earlier, Gray (19) had found in the cat CB perfused *in situ* with a cell-free solution that acetazolamide diminished the speed of the chemosensory responses to acid hypercapnia. Our results obtained with the superfused and perfused cat CB preparation are consistent with his observation. However, Gray did not find any significant overshoot in his normal responses to acid hypercapnia, but in our preparation, by contrast, we always found a fast overshoot of the initial response to CO_2, which was eliminated by methazolamide. Thus, the effects of CA inhibition on the speed and magnitude of the initial responses to CO_2 are greater and clearer in the *in vitro* perfused cat CB preparation. The difference is probably due to the fact that the hypercapnic stimulus flows past the CB promptly in our preparation as some arterial vessels (*e.g.*, ascending pharyngeal) are kept open, so that the CB receives the stimulus suddenly. We also had HEPES, a buffer other than CO_2–HCO_3^- present, and Gray remarked that he had seen adaptation to CO_2 in the presence of another buffer, Tris.

Methazolamide produced a significant decrease in the basal chemosensory discharge in the perfused CB in the presence of CO_2 at constant pH. This indicates that CA inhibition somehow diminishes the intensity of the steady-state stimulus at the cellular level, and suggests that the catalyzed production of H^+ in the presence of CO_2–HCO_3^- contributes to maintain an acidic steady-state pH_i inside the cells (Fig 4). Several types of cells, including rat glomus cells (6), do show intracellular alkalinization during CA inhibition. In the absence of CO_2–HCO_3^-, the effect of CA inhibition on baseline chemosensory activity was minimal (26, 29), presumably because endogenous CO_2 had little effect and the Cl^-–HCO_3^- exchanger mechanism was less effective (5, 40, 51).

CAROTID CHEMOSENSORY RESPONSE TO HYPERCAPNIA

CO_2 is soluble and easy permeates across the biological membranes (2) and, consequently, it equilibrates within all regions of the CB. This is evident from the rapid chemosensory response to high CO_2. The response to acidic hypercapnia consists of three phases: an initial overshoot, an approach to the steady-state and then adaptation. If the effect of a sudden CO_2 increase depends on the catalyzed intracellular production of H^+ and the discharge parallels the increasing $[H^+]_i$, the rise of the chemosensory response should be delayed and reduced by CA inhibition. This can explain the elimination of the typical overshoot of the response to the onset of the hypercapnia. The initial overshoot of the response to CO_2 and the subsequent decline or adaptation could be the result of an initial increase of $[H^+]_i$, which is not maintained because the elevated acidity stimulates mechanisms for the extrusion of the acid from the cells (2, 5, 40, 51), resulting in a subsequent decline in the $[H^+]_i$ and so also in the chemosensory activity. The persistence of a reduced late response during maintained acidic hypercapnia (respiratory acidosis) after CA inhibition (4, 20, 21, 26, 29) could be due to a slow uncatalyzed hydration of CO_2, but also to a slow equilibration of the intracellular space with the increased extracellular acidity.

The above idea is supported by the fact that metabolic acidosis stimulates the carotid chemosensory discharge *in situ* (47) and *in vitro* (11), and the pH_i of the glomus cells shows a high dependency on the extracellular pH. In fact, Buckler *et al.* (7) and Wilding *et al.* (64) found in rat glomus cells a linear

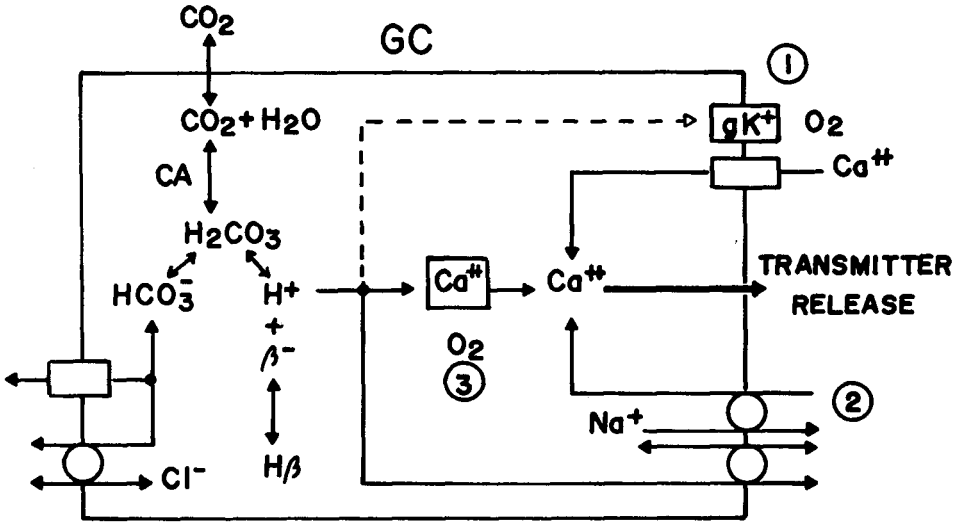

Fig 4. Schematic diagram of proposed mechanisms for CO_2–H^+ chemotransduction operating in the glomus cell (GC) of the CB. Intracellular acidosis produced by enzymatic CO_2 hydration would increase free $[Ca^{2+}]_i$ in the GC, and that in turn would initiate transmitters release by the following mechanisms: 1) Reducing the glomus cell K^+–PO_2 conductance, the resulting depolarization would raise free $[Ca^{2+}]_i$ by opening voltage-gated Ca^{2+} channels. 2) Increasing $[Na^+]_i$ through H^+–Na^+ exchanger activation evoked by the increased $[H^+]_i$; a large elevation of $[Na^+]_i$ may increase free $[Ca^{2+}]_i$ by reversing the normal function of the Na^+–Ca^{2+} exchanger. 3) Inducing Ca^{2+} release from an intracellular pool, which can be also dependent of PO_2. Cellular alkalinization induced by large H^+ extrusion or by reduced HCO_3^- extrusion should decrease free $[Ca^{2+}]_i$ and the ensuing chemosensory discharges. Dashed line, inhibitory effect; solid line, excitatory effect.

relationship between pH_i and extracellular pH (pH_e), with the slope (pH_i/pH_e) of the relationship in the absence of CO_2–HCO_3^- being 0.63 and 0.85, respectively. In the presence of CO_2–HCO_3^-, this slope was little changed to 0.68 and 0.82.

We do not understand exactly how an increase of intracellular CO_2 and $[H^+]_i$ might augment the chemosensory discharge. It is known that a rise of $[H^+]_i$ increases $[Na^+]_i$ through H^+–Na^+ exchanger mechanisms, and a large increase of $[Na^+]_i$ can in turn augment $[Ca^{2+}]_i$ by reversing the normal function of the Na^+–Ca^{2+} exchanger. Recently, Rigual *et al.* (50) proposed such an hypothesis to explain the dopamine release from the CB evoked by acid and hypercapnic stimuli. According to this idea, acidosis would increase free $[Ca^{2+}]_i$ in the glomus cells, and that in turn would initiate the release of transmitters (see Fig 4). This idea was supported by the observation that the application of acetazolamide to the superfused cat CB *in vitro* reduced both the chemosensory response and the release of dopamine elicited by a high CO_2–H^+ stimulus (50). However, the presence of a Na^+–Ca^{2+} exchanger in glomus cells has not yet been demonstrated and a rise of $[Ca^{2+}]i$ in response to CO_2–H^+ might be produced by other mechanisms. For instance, CA is involved in intracellular Ca^{2+} mobilization in muscle cells (17) and a similar mechanism could operate in the glomus cells (Fig 4). The initial peak of the CO_2 response may be related to a large but localized transient rise of $[H^+]_i$ in some specific sites containing CA and high $[Ca^{2+}]$. Also, it is known that an increase of intracellular $[H^+]$ may depolarize the glomus cells (22, 24), which in turn would open voltage-gated Ca^{2+} channels. Therefore, intracellular acidity might increase the $[Ca^{2+}]_i$ by several ways. New experiments using Ca^{2+} and H^+ fluorescent dyes seem to be crucial for determining the relationships between CO_2–H^+, CA and $[Ca^{2+}]_i$ in the glomus cells.

CAROTID CHEMOSENSORY RESPONSE TO HYPOXIA

Experiments performed *in vitro* showed that the chemosensory response to hypoxia of the

cat CB to hypoxia was delayed by CA inhibition (26, 28, 29). An action that diminished the chemosensory response to CO_2 could attenuate the response to hypoxia also in several ways. A delay in the response to hypoxia after application of a CA inhibitor is consistent with an attenuation of the response to CO_2–H^+. A reduction in the speed of the response to hypoxia could be attributed to a diminished acidity of the chemoreceptor cell. In the nominal absence of CO_2–HCO_3^-, it was indeed found that the pH_i of the glomus cells was more alkaline (6, 7, 31, 64), due to blockade of the acid loader function of the Cl^-–HCO_3^- exchanger (6). Cellular alkalosis might well raise the threshold of the hypoxic response and diminish sensitivity to a low O_2 stimulus (12, 33). In fact, metabolic alkalosis in situ and *in vitro* decreased the responsiveness of the CB to hypoxia (12) and this effect is likely to have been caused by a glomus cell alkalinization (6, 7, 64).

The mechanisms by which the O_2 and CO_2 stimuli interact (see 12 for review) are not completely known; however, there is some evidence of how this may happen. If transmitters are released by these stimuli, as it seems to be the case (12, 14, 50), it is possible that the presence of CO_2–HCO_3^- in the cellular environment would augment the release of the transmitter(s) during hypoxia (Fig 4). In studies with isolated glomus cells in a medium nominally free of CO_2–HCO_3^-, hypoxia diminished the membrane K^+ conductance (38, 39, 45, 46, 55) and increased the intracellular free $[Ca^{2+}]_i$ (3). These results are consistent with the finding that the hypoxic chemosensory response does not require the participation of external CO_2–HCO_3^-. Nevertheless, the presence of external CO_2–HCO_3^- does strongly potentiate the speed and magnitude of the hypoxic response (25, 35). Shirahata and Fitzgerald (56) stated that CO_2–HCO_3^- is essential for hypoxic chemoreception, because they did not observe in their cat CB *in situ* preparation any significant chemosensory response to hypoxia in the nominal absence of CO_2–HCO_3^- from perfusate. However, several reports have shown that hypoxia does stimulate the carotid chemoreceptors *in vitro* in the nominal absence of CO_2–HCO_3^- (see 12 for review).

The basic mechanism of hypoxic chemoreception is not completely known (12, 14). In the context of the present review, the acid hypothesis of chemoreception deserves some discussion. According to Torrance, carotid chemoreception to O_2 and to CO_2 is mediated by a common mechanism of cellular acidification (20). He proposed that a HCO_3^- pump controls the $[H^+]_i$ as a reciprocal of PO_2. However, Lahiri, Mulligan and colleagues (42, 57) presented evidence for two separate chemosensing sites for O_2 and CO_2. In fact, they demonstrated that the application of metabolic inhibitors abolished the carotid chemosensory response to low O_2 but the response to CO_2 persisted and was even augmented, showing a strong adaptation after the initial peak. These results suggested that the response to hypoxia and adaptation of the response to CO_2 are not causally linked. However, cellular acidosis during hypoxia could be produced by some other mechanism. Delpiano and Acker (8), using extracellular microelectrodes, found that hypoxia does cause extracellular acidosis in the cat CB and they proposed that hypoxia increases the glycolytic production of acid, which could somehow mediate the chemosensory response to hypoxia. However, the extracellular acidosis that is observed during hypoxia could be the result of an increased extrusion of H^+ from the cells even though there is not a true intracellular acidification.

The above interpretation is supported by the fact that Garcia-Sancho *et al.* (18), using 5,5 dimethyl-2,4-oxalidinedione as a pH indicator, were unable to find any pH change in the whole rabbit CB during severe hypoxia. Recently, Iturriaga *et al.* (31), using an optical method (BCECF fluorescent dye) to study pH_i changes in the whole cat CB *in vitro*, found that hypoxia –unlike isohydric and acid hypercapnia or sodium acetate– did not reduce the pH_i when it stimulated the chemosensory discharge. There were no obvious fluorescence changes during hypoxia. Likewise, Biscoe *et al.* (3) did not detect any pH_i changes in isolated rabbit glomus cells during application of 2 mM cyanide. Recently, Eyzaguirre and

colleagues (23) used H^+-sensitive microelectrodes and showed that hypercapnia reduced pH_i in 90% of the isolated rat glomus cells studied, whereas relative hypoxia produced by a change of the superfusate medium –from one equilibrated with 50% O_2 to one equilibrated with room air– caused inconsistent changes in the glomus cell pH_i, some cells showing acidification and others alkalinization. Making measurements of pH_i with the fluorescent dye BCECF in isolated adult rat glomus cells, Wilding *et al.* (64) arrived to the same conclusion. These results taken together make it difficult to accept the suggestion that the hypoxic response is initiated by $[H^+]_i$ changes (20, 61), but they are not against the idea that O_2–CO_2 stimulus interaction may depend on the pH_i setting. Thus, the balance of evidences does not support the hypothesis that hypoxic chemoreception is initiated by intracellular acidosis.

Several electrophysiological studies using patch clamp whole-cell recording techniques have shown that rat and rabbit glomus cells have an outward voltage-activated K^+ conductance which is reduced by hypoxia (38, 39, 45, 46, 55). Low PO_2 reversibly reduces the K^+ current evoked by an imposed depolarization. This suggests that the response to hypoxia is initiated by a depolarization caused by the low PO_2. The resulting depolarization could raise the intracellular free $[Ca^{2+}]$ by opening voltage-gated Ca^{2+} channels (38, 39). It has also been found that the K^+ current is reduced by extracellular and intracellular acidification (45, 46, 55). Stea and Nurse (54) reported that the K^+ conductance is decreased in the presence of CO_2–HCO_3^- and the magnitude of this decrease is similar to that produced by intracellular acidosis (55). Therefore, it is possible that the intracellular acidification caused by high CO_2 and mediated by the action of CA may modulate the hypoxic electrophysiological response (Fig 4).

REFLEX VENTILATORY RESPONSES

Inhibition of CA in the whole animal results in hyperventilation and a large fall in the alveolar PCO_2 (9, 59, 60, 63). Hyperventilation probably develops because there is a retention of CO_2. Therefore, an acidosis in the tissues may stimulate the central chemoreceptors. Experiments performed on cats (59, 60) and humans (58) showed that the acute administration of acetazolamide reduced the magnitude of the reflex ventilatory responses to hypercapnia and isocapnic-hypoxia, and suggested that the carotid chemosensory responses are depressed. However, a major difficulty in these experiments is that the CO_2–HCO_3^- disequilibrium produced in the red blood cells by the inhibition of CA produces an apparent arterial-alveolar PCO_2 gradient, which means that the PCO_2 and pH levels in the CB are unknown.

The effects of CA inhibition on ventilation in the intact animal may arise from several sites of action, because CA is present in the central nervous system, in the CB and in the red blood cells. Nevertheless, the results obtained *in vitro* (26, 28, 29) support the proposal that CA inhibition decreases the carotid chemosensory output *in situ*. We can expect that the major effects of acute CA inhibition at the CB level should be exerted on the baseline carotid chemosensory discharge and on the speed and magnitude of the initial responses to hypercapnic and hypoxic stimuli. These fast physiological reflexes will be affected without an effective CA function in the CB.

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