Rat carotid body chemosensory discharge and glomus cell HIF-1α expression in vitro: regulation by a common oxygen sensor

Arijit Roy,1 Santhosh M. Baby,1 David F. Wilson,2 and Sukhamay Lahiri1

1Department of Physiology and 2Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

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The carotid body (CB) consists primarily of glomus cell units comprising 5–10 cells innervated by afferent fibers that have cell bodies in the petrosal ganglia. These are on the path to brain stem neurons responsible for the respiratory and cardiovascular reflexes (23). Glomus cells are known to possess oxygen sensors (10), and, within a breath of hypoxic mixture and subsequent fall of arterial oxygen pressure, the afferent nerves begin to show an increase in discharge rate. CO is competitive with O2 in the CB, and the afferent electrical activity of the carotid sinus nerve (CSN) increases with increasing CO if the oxygen pressure is kept constant (18, 44). The onset of this CO effect has been shown to be rapidly and fully reversed by bright white light (44). This pattern is a very distinctive characteristic of CO compounds of reduced iron porphyrin. The CO-iron porphyrin compounds were first shown to hyperpolarize (increase K+ current) on glomus cell membrane, i.e., it increases whole cell K+ conductance could control the membrane potential of the nerve endings (21). CO is, however, reported to block the hypoxia-induced inhibition of whole cell K+ current and depolarization on glomus cell membrane, i.e., it increases whole cell K+ current with a leftward shift of the reversal potential (25, 34). As such, the reported CO-mediated changes in cell membrane conductance cannot be responsible for the excitation of the chemosensory discharge. Hypoxia-inducible factor 1α (HIF-1α) is present in the glomus cells (3, 4). Recently, CB chemosensory response has been linked to the glomus cell transcription factor HIF-1α and the level of HIF-1α shown to increase after in vitro exposure of the CB to hypoxia (3). The increased in HIF-1α during hypoxia characterizing the role of heme proteins (e.g., biological oxides) in oxygen metabolism. Warburg (41), for example, first demonstrated that inhibition of cellular oxygen consumption by CO was reversed by light. Warburg and Negelein (42) then measured the wavelength dependence of this reversal and obtained the spectrum of the CO compound of cytochrome a3, showing that this heme protein is responsible for most of the oxygen consumption by yeast and mammalian cells.

More recently, the light-induced reversal of the CO-induced increase in oxygen sensory activity in an isolated perfused-supersaturated CB preparation (16) was used to identify the oxygen sensor of the CB (43, 44). When monochromatic light of different wavelengths but at equal intensities is used, the extent of the reversal is dependent on wavelength of light, with greatest efficacy at 432 ± 2 and 590 ± 2 nm. Light at 432 nm was six to seven times more effective than light at 590 nm. These spectral characteristics show that the inhibitory CO complex is that of mitochondrial cytochrome a3, as first described by Warburg and Negelein (42). Lahiri et al. (20) reported that, in the dark, high CO diminished O2 consumption in the CB, and this correlated with the increase in chemosensory activity. Both of these effects were reversed by bright (white) light, consistent with their mediation by cytochrome a3. Mulligan and Lahiri (29) also provided evidence for a role of mitochondrial respiratory chain in CB chemosensory activity by showing that there was transient stimulation of CSN activity when inhibitors of mitochondrial electron transport chain were added [also see Wyatt and Buckler (46)].

CO, in addition to its effect on mitochondria, has been reported to hyperpolarize (increase K+ current) the glomus cell membrane in normoxia (5, 24). The implication is that CO, under normoxia, binds to a heme protein that is attached to an ion channel and controls its conductance. The changes in conductance could control the membrane potential of the nerve endings (21). CO is, however, reported to block the hypoxia-induced inhibition of whole cell K+ current and depolarization on glomus cell membrane, i.e., it increases whole cell K+ current with a leftward shift of the reversal potential (25, 34). As such, the reported CO-mediated changes in cell membrane conductance cannot be responsible for the excitation of the chemosensory discharge.

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is regulated at the HIF-1α mRNA level as determined by O₂ concentration in the 0–95% range (36). Kline et al. (17) reported that mice partly deficient in HIF-1α showed a significantly diminished chemosensory response to hypoxia. Both the CB CSN response and the increase in HIF-1α with hypoxia are known to be suppressed by inhibitors of mitochondrial respiration (2, 4, 12, 27). As a result, it appears that there could be a link between HIF-1α and chemosensory response in CB, but the nature of this link was not established.

In the present study, CO has been used as an experimental tool to examine the relationship of the hypoxia-induced increase in HIF-1α and the CSN activity of the CB. It is shown that CO induces a hypoxia-like response for both and that the wavelength dependence of the light-induced reversal of CO effects are the same. It is concluded that the oxygen sensor for hypoxia-induced increases in glomus cell HIF-1α expression and CB chemosensory response is the same. There are, however, substantial temporal differences between the two responses: the rate of change in the CSN response is maximal in <2 s, whereas HIF-1α induction in the CB by either hypoxia or Fe²⁺ chelation becomes significant in 2 min and maximal in ~10 min (37). In cells, Huang et al. (14) reported that, when HIF-1α was induced in cells by hypoxia and then the cells were given a step increase to 21% oxygen, the half-time for the decrease was <5 min, but the time to maximal change was >5 min. Thus, although cytochrome a₃ of mitochondrial respiratory chain is the oxygen sensor for both responses, they have different physiological roles. The CSN activity provides the very rapid response necessary for controlling breathing on a breath-to-breath basis, whereas HIF-1α induction contributes to the intermediate and long-term adaptation of cells and tissue to chronic changes in oxygen pressures [for reviews, see Semenza (38, 39) and Guzy and Schumaker (11)].

**MATERIALS AND METHODS**

All of the animal experiments were carried out by investigators trained to handle rats and the experimental procedures were given prior review and approval by the local IACUC committee. At the end of each experiment, the rat was euthanized according to guidelines established by the AVMA Panel on Euthanasia.

**CB perfusion and superfusion.** The carotid bifurcation was prepared from anesthetized male rats (200–225 g) for in vitro perfusion essentially as described previously (35). Single-pass perfusion and superfusion were established by hydrostatic pressures of 90–100 Torr. The perfusate-superfusate was modified Tyrode solution containing (in mM) 112 NaCl, 4.7 KCl, 2.2 CaCl₂, 1.1 MgCl₂, 20.5 NaHCO₃, 5.0 HEPES, 5.5 glucose, and 20.8 sodium glutamate, as well as 4 g/l dextran (molecular weight = 742,000) and 0.25 mM l-ascorbic acid at pH 7.4. The perfusate was equilibrated with either 21% oxygen-5% CO₂, remainder nitrogen or a mixture of 21% oxygen-5% CO₂ remainder CO. The temperature in the perfusion chamber was maintained at 36.5 ± 0.5°C. Paraffin oil was layered over the superfusate, and the whole desheathed sinus nerve was placed on a platinum electrode for recording of chemosensory discharge.

**Measurement of the light-induced reversal of the CO effect (photocchemical action spectrum).** The wavelength dependence (spectral response) of the light-induced reversal of the CO-induced increase in CSN activity of the CB is that of the CO complex of reduced cytochrome a₃ (43, 44). The spectrum was measured as the light-induced changes in CSN for the isolated CB perfused with buffer equilibrated with a mixture of CO (PCO of ~350 Torr) and oxygen (PO₂ of ~130 Torr). Details of the experimental procedure were the same as described earlier (42). The monochromatic illuminating light was obtained by passing the light of a tungsten-halogen light through a monochromator into a light guide. The light from the light guide was focused on an ~1-mm area of the CB preparation and centered as best as possible. The required intensities of monochromatic light were relatively high, and this requirement was further increased depending on the preparation geometry, light scattering, and other factors. Five different wavelengths of monochromatic light, ranging from 430 to 610 nm, were used to determine the wavelength dependence of the CO reversal effect.

**Glomus cell preparation.** The glomus cells were separated enzymatically as described previously (3). Briefly, CBs (n = 20) were surgically removed from anesthetized rats, cleared of connective tissue, and digested with 0.2% collagenase P (Roche Diagnostics) and 0.1% trypsin (Sigma, St. Louis, MO) in a Ca²⁺- and Mg²⁺-free phosphate buffer for 30 min at 37°C. The digested tissue was transferred to a solution of growth medium, triturated with a fire-polished Pasteur pipette, washed, and plated on 18-mm coverslips in a Petri dish. The growth medium contained FBS plus streptomycin, penicillin G, and insulin. The Petri dishes containing the separated cells were left undisturbed for a period of 24–30 h in a humidified incubator (37°C, circulated with 5% CO₂ and air) for cells to adhere on to the coverslip. In all cell measurements, tyrosine hydroxylase was measured to verify that the cells were glomus cells. Glomus cells are the only cells in the CB that contain tyrosine hydroxylase.

**Superfusion of the isolated glomus cells.** Coverslips adherent cells were in a closed chamber and superfused with HEPES-buffered medium (pH ~7.3): 139 mM NaCl, 4.9 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 10 mM HEPES, 10 mM glucose, and 250 μM l-ascorbic acid. Glomus cells were superfused with either normoxic (P O₂ of ~130 Torr) or normoxic with CO (PCO of ~350 Torr; PO₂ of ~130 Torr) medium for 45–50 min at 37°C. The high-oxygen pressure in the medium and the continuous refreshing of the medium around the cells ensured that 1) when the superfusion medium was changed to one with CO, the PO₂ in the medium and at the cell surface were unchanged and near 130 Torr and 2) when superfusing with CO, the CO-to-O₂ ratio was ~3, low enough to cause only a partial decrease in mitochondrial respiratory capacity. At the end of the superfusion period, the glomus cells were fixed in 4% ice-cold paraformaldehyde. They were later used for immunofluorescence measurements of HIF-1α and tyrosine hydroxylase.

**HIF-1α and tyrosine hydroxylase immunofluorescence.** Adherent cells were fixed in 4% paraformaldehyde in 0.01 M PBS (pH 7.3) for 10 min, washed three times with PBS, permeabilized with 0.05% Triton X-100 in PBS for 5 min, and rinsed again three times with PBS. Nonspecific binding was blocked by incubating with 10% FBS in PBS for 1 h at room temperature. The cells were then incubated with anti-HIF-1α at 1:200 (rabbit IgG, epitope mapping with amino acids 329–530 of HIF-1α of human origin; Santa Cruz Biotechnology) or anti-tyrosine hydroxylase antibody at 1:500 (mouse IgG; Sigma) at 4°C overnight in a humidified chamber. After incubation with primary antibodies, cells were washed in PBS for 10 min and incubated in secondary antibodies for 1 h at room temperature. These consist of goat anti-mouse IgG conjugated to Texas red (1:200; Chemicon International) and goat anti-rabbit IgG conjugated to FITC (1:200; Jackson Immunoresearch Laboratories, West Grove, PA). After a thorough rinse with PBS, cells were covered with a photobleaching reagent (Mowiol; Calbiochem, La Jolla, CA) containing DABCO (2,2,2)-nitrobenzylamine and iminodiace (see below). Controls were achieved either by omitting primary antibodies or by incubating cells with goat serum instead of primary antibodies. No nonspecific immunofluorescence was observed in the controls.

**Quantification of HIF-1α and tyrosine hydroxylase by immunofluorescence.** Quantification of the changes in immunolabeling of HIF-1α was by a modification of the original method described previously (3). Briefly, isolated, cultured glomus cells were viewed with a Nikon Eclipse TE300 fluorescence microscope (×60, ×100 oil
immersed) and equipped with an optical filter changer (Lambda DG-4; Sutter Instruments, Novato, CA). Fluorescence excitation was accomplished with a mercury lamp (150 W) fiber optic light source and appropriate filter sets. To prevent photobleaching of the fluorescent preparation, a neutral density filter (ND 0.3; Chroma Technology) was used to attenuate 50% of the light intensity. The HIF-α and tyrosine hydroxylase immunofluorescence intensity images were acquired with 12-bit digitally cooled charged-coupled device camera (ORCA 100; Hamamatsu), using graphics control software (Metamorph Imaging System, Universal Imaging). Images of glomus cells were digitally marked, and the pixel intensities were calculated. The background regions outside the cells were also digitally marked, and the average pixel intensity was subtracted from each cell image. For each cell, the average intensity was determined for the entire cell area, and this was used as a measure of the level of HIF-1α for that cell.

Statistical analysis. The summary data in graphs are for the averages over entire cells and are presented as means ± SE and number of experiments for each condition (normoxia, hypoxia, and hypoxia plus mitochondrial inhibitors). Results were analyzed by one-way ANOVA (SigmaStat, Jandel Scientific). Differences were considered statistically significant when $P < 0.05$.

RESULTS

The effect of CO administration on the afferent electrical activity of the in vitro rat CB both in the dark and when illuminated with monochromatic light of different wavelengths.

When the perfusate was switched from normoxic medium without CO to one containing CO (P_{CO} of ~350 Torr; P_{O2} of ~130 Torr) in the dark, CSN discharge (impulses/s) increased very rapidly (1–2 s) from a basal average value of 60 to 420 impulses/s. This increase in neural activity was completely reversed by illumination with the CB with bright white light (Fig. 1). The above procedure was repeated with experimentally indistinguishable results. The response to turning the light on or off was rapid and reproducible. On the other hand, as long as CO-containing buffer was used and the light was off, the neural activity remained essentially constant and at a high level.

In a separate experimental preparation, CB perfused with CO (in normoxia) was exposed to monochromatic light for which the wavelength could be switched among the wavelengths 430, 450, 550, 590, and 610 nm. Previously, Wilson et al. (44) showed, in cat CB, that the wavelength dependence of the reversal of the CO effect on afferent neural activity had well-defined maxima at near 430 and 590 nm.

Fig. 1. Neural discharge recorded from the carotid sinus nerve (CSN) of an isolated perfused rat carotid body (CB). The CB was perfused with a normoxic medium (P_{O2} of ~130 Torr) and then switched to a medium containing the same oxygen pressure and CO (P_{CO} of ~350 Torr). Introduction of the CO caused immediate excitation of the CB in the dark, as indicated by the increase in CSN discharge. Illumination with a bright white light while perfusing with CO-containing medium returned the CSN activity to the pre-CO baseline level. The light-induced suppression of CSN activity could be repeated several times with reproducible results.

Fig. 2. Photosensitivity of the CSN discharge of the rat CB perfused with normoxic medium containing CO (P_{CO} of ~350 Torr). A: after an initial period of perfusion in normoxic medium without CO, the perfusion medium was changed to one with CO, with the oxygen pressure remaining the same (P_{CO} of ~350 Torr; P_{O2} of ~130 Torr). The afferent CSN activity increased markedly (lights off); after it stabilized, the CB was illuminated with monochromatic light with wavelengths of 430, 450, 550, 590, or 610 nm for brief periods. The periods of illumination were separated by periods of dark (lights off) sufficient for the CSN activity to return to the steady state. The periods with the lights on were all of equal length, as were the intervening periods with the light off. Much greater light-induced suppression of the CSN activity occurred for wavelengths of 430 and 590 nm than for the other wavelengths. B: bar graph showing the relative efficacy of light of the different wavelengths in suppressing the CO-induced increase in CSN activity. * $P < 0.001$, CSN activities at 450, 550, and 610 nm compared with 430 and 590 nm.
Effect of CO administration on expression of HIF-1α in rat CB glomus cells in the dark and when illuminated with monochromatic light of different wavelengths. Figure 3A shows images of the immunofluorescence of glomus cells stained for HIF-1α and tyrosine hydroxylase, along with an overlay of the two images to show colocalization. To compare the changes in

HIF-1α induced by adding CO with those induced by hypoxia, glomus cells were superfused for 45 min with either normoxic medium (PO_2 of ~130 Torr) (row 1) or a medium with low oxygen (1% O_2) (row 2). The low-oxygen medium resulted in the well-established hypoxia-induced increase in HIF-1α due to stabilization of HIF-1α and an increase in tyrosine hydrox-

Fig. 3. Fluorescence microscope images showing the immunofluorescence intensities of HIF-1α (left) and tyrosine hydroxylase (TH) in glomus cells (middle) and the extent of their colocalization (right). A, row 1: low-fluorescence intensities show the low levels of HIF-1α and TH in cells incubated in normoxic media. Row 2: glomus cells incubated in medium containing 1% O_2 for 45 min show a marked increase in the immunofluorescence due to HIF-1α, consistent with the hypoxia-induced increase in HIF-1α.

Row 3: incubation of the cells in a medium containing CO (PCO_2 of ~350 Torr) and O_2 (PO_2 of ~130 Torr) for 45 min illuminated with bright white light resulted in levels of HIF-1α similar to those for normoxic medium without CO. Row 4: incubation in CO-containing normoxic medium, but in the dark, resulted in substantial increases in HIF-1α. Magnification = ×600. Bar = 15 μM. B: bar graph showing the levels of HIF-1α in the glomus cells (immunofluorescence intensity in arbitrary units). There was significantly higher (P < 0.001 compared with normoxia) HIF-1α when the glomus cells were incubated with CO in the dark than when they were incubated in either normoxic medium without CO or CO-containing medium illuminated with bright white light. The increase during incubation with CO in the dark was equivalent to that during incubation in a medium equilibrated with 1% oxygen.
The ability of light to block the CO-induced increase in HIF-1α in glomus cells is summarized in Fig. 3. The ability of light to block the CO-induced increase in HIF-1α in glomus cells is summarized in Fig. 3B. There was significant accumulation of HIF-1α in cells superfused with CO in the dark but not when they were illuminated with white light during the superfusion period.

In a separate set of experiments, glomus cells were superfused with CO (PCO of ~350 Torr)-containing normoxic (PO2 of ~130 Torr) medium for 45 min while cells were illuminated with monochromatic light having wavelengths of 430, 500, 590, and 610 nm (Fig. 4A). When glomus cells were incubated in normoxic medium (no CO), illumination during incubation had no effect on the levels of HIF-1α or tyrosine hydroxylase (row 1). In contrast, if the glomus cells were kept in the dark while superfused with normoxic medium containing CO, the level of HIF-1α immunofluorescence greatly increased compared with normoxic controls. This CO-induced increase was not obviously affected by illumination when the wavelength of the monochromatic light was 500 nm (row 3) or 610 nm (row 4). In contrast, when illumination was with light of 430 nm (row 2) or 590 nm (row 4), the HIF-1α immunofluorescence was markedly lowered, to only slightly above the normoxic controls. Although the increases in HIF-1α during illumination at 430 and 590 nm light were small, they are statistically significant compared with cells superfused with medium without CO (Fig. 4B) or with CO but illuminated with bright white light.

**DISCUSSION**

General agreement has not been reached regarding the mechanism by which the CB senses the oxygen pressure and uses this information to control its afferent CSN activity, although several hypotheses have been put forward (1, 23, 33, 40, 44). The CB has several other biochemical and physiological responses to altered oxygen pressure in the cellular environment, and one of these is to increase the levels of HIF-1α (3). The present study compared the responses of rat CB CSN activity and glomus cell HIF-1α to inclusion of CO in the medium. Both the CB sensory activity and glomus cell HIF-1α increased when the incubation medium contained CO, despite the fact that the oxygen pressure in the medium was not changed. This observation is consistent with the addition of CO to the medium being functionally equivalent to decreasing the oxygen pressure.

Because it has been shown (19, 44) that light can rapidly and reversibly block the increase in afferent neural activity induced by addition of CO, experiments were designed to determine the efficacy of light on the CO-induced increase in HIF-1α. The data show that CO induced marked increases in both the afferent CSN activity and HIF-1α, and bright white light was fully effective in reversing both responses. Moreover, when monochromatic light was used, both responses showed the same dependence on the wavelength of light used for illumination. This is strong evidence that the CO-induced increase in afferent neural activity of the CB and in HIF-1α levels in the glomus cells are mediated by the same oxygen-sensing mechanism. The only known pigment with the measured properties (CO competitive with O2, CO effect reversed by moderate light intensities, CO complex having absorption maxima at 430 and 590 nm) is the CO complex of reduced cytochrome c3 of the mitochondrial respiratory chain.

Light has also been reported to increase the excitatory activity of the CB when that activity has been suppressed by "severe hypoxia" in the presence of CO [see Lahiri et al. (21)]. As the CO-to-O2 ratio is increased, the CSN activity increases to the maximal value for very low oxygen and then, as synthesis of ATP by the mitochondria fails to meet the ATP requirement for neural discharge, decreases again. At these high CO-to-O2 ratios, where CSN activity is suppressed because of a lack of ATP, illumination with white light can cause an increase in afferent CSN activity. The light, by relieving the inhibition of cytochrome c-oxidase by CO, increases ATP synthesis by the mitochondria. With increased ATP availability, the neural activity increases, but only for as long as the ATP is increased (as long as the light is on).

A reasonable hypothesis for the mechanism of oxygen sensing and activation of the sensory responses and the mechanism of CO interaction with the oxygen sensor is as follows. Mitochondrial oxidative phosphorylation maintains the cellular energy state ([ATP]/[ADP][Pi], where brackets indicate concentration) at a nearly constant (steady-state) value under normoxic conditions. When the oxygen pressure decreases or CO increases (see below), there is a decrease in energy state and this decrease (possibly through increased AMP levels) results in increasing intracellular calcium concentration through release from internal calcium stores and by influx of extracellular calcium (20, 28). Increased intracellular calcium causes increased release of neurotransmitters such as dopamine (6) and ATP (30), increasing afferent neural activity. The increase in HIF-1α could be mediated by the same sensor system because a decrease in cellular energy state would activate the cellular stress response system. In the case of HIF-1α, the response is slower because of the time required for complex regulatory functions at the level of alterations in protein stability and gene expression.

The effects of CO can be understood from its known reaction with the reduced form of mitochondrial cytochrome c3, where it is competitive with respect to oxygen. In the dark, the increase in pressure of CO at constant oxygen pressures is the metabolic equivalent of decreasing the pressure of oxygen in the absence of CO. Increasing CO would, like decreasing O2, cause a decrease in oxygen consumption (19, 20) and ATP production by the mitochondrial oxidative phosphorylation. The resulting decrease in cellular energy state would signal for induction of HIF-1α and other stress-response elements.

There have been other effects of CO reported for the CB and glomus cells. Glomus cell membranes have been reported to hyperpolarize with high CO due to reversal of hypoxia-induced suppression of the background leak potassium current (5) and calcium-activated potassium current (34). These CO effects were reported for CO together with hypoxia and were not light sensitive, indicating they are not involved in the CO-induced increase in chemosensory discharge or induction of HIF-1α described for this study. Huang et al. (14) reported that, in...
Fig. 4. Fluorescence images showing the wavelength dependence of the effect of light on CO-induced accumulation of glomus cell HIF-1α (left) and TH (middle). A: colocalization of HIF-1α and TH immunofluorescence is shown on right. Row 1: when the cells were incubated in normoxic medium without CO and illuminated with bright white light, the level of HIF-1α immunofluorescence was minimal. Row 2: glomus cells incubated while illuminated with monochromatic light at 430 nm in a medium with CO show only slightly increased HIF-1α compared with control. Row 3: glomus cells incubated in CO-containing medium and illuminated with 550-nm light show markedly increased HIF-1α. Row 4: glomus cells incubated in CO-containing medium and illuminated with 590-nm light show only slight increases in HIF-1α fluorescence, much like illumination with 430-nm light (above). Row 5: glomus cells incubated in CO-containing medium and illuminated with 610-nm light have markedly increased HIF-1α immunofluorescence. Magnification = ×600. Bar = 15 μM. B: wavelength dependence of the effect of light on the CO-induced increase in HIF-1α in glomus cells. The level of HIF-1α immunofluorescence in glomus cells was significantly higher (**P < 0.001) when they were illuminated with 500- or 610-nm light than when they were illuminated with 430- or 590-nm light. The latter values were still significantly higher (*P < 0.05) than for normoxic controls illuminated with white light.
Hep3B cells incubated under a low-oxygen gas phase, CO decreased the level of HIF-1α protein. However, these authors did not determine the oxygen pressure in the cellular environment, making this effect hard to interpret (see further discussion below).

The HIF-1α response when glomus cells are incubated in normoxic media with CO is much slower than the neural response (~45 min vs. <2 s). In the present paper, we have demonstrated that, despite the difference in response rate, the CO-induced increase in glomus cell HIF-1α, like the CO-induced increase in the CSN activity of the isolated CB, can be suppressed by light. Furthermore, using monochromatic light, we have shown that induction of HIF-1α by CO was more strongly suppressed by monochromatic light at wavelengths of 430 and 590 nm than at 550 and 610 nm (Fig. 4). This spectral result indicates that mitochondrial cytochrome a$_3$ was the oxygen sensor that mediated the observed HIF-1α response.

In the present experiments, illumination with monochromatic light with wavelengths of 430 or 590 nm did not entirely abolish the increase in HIF-1α, whereas illumination with white light did. This is not surprising because the white light was obtained by direct illumination by the light source, removing only the infrared (heat) component. To obtain monochromatic light, this white light beam was passed through a monochromator and separated into its component colors (wavelengths). The intensity of the monochromatic light was necessarily of lower efficacy than the white light from which it was isolated.

In attached cell cultures incubated in medium covered with a low-oxygen pressure gas phase, it has been reported that addition of high concentrations of mitochondrial inhibitors, such as CO and NO, results in suppression of the HIF-1α increase (7, 9, 12, 15). Hagen et al. (12) suggested that the mitochondrial inhibitors block the hypoxia-induced stabilized HIF-1α by redistribution of the oxygen within the cell, whereas Huang et al. (15) proposed it was due to displacement of O$_2$ from oxygen-dependent degradation domain of HIF-1α. Callapina et al. (7) suggested it resulted from increased production of oxygen radicals. Doege et al. (9), however, measured the oxygen pressures in the cellular environment and showed that, if the oxygen diffusion gradient generated by cellular oxygen consumption was eliminated, the mitochondrial inhibitors no longer induced a decrease in HIF-1α. They concluded “that reduction of oxygen consumption reduces the O$_2$ gradient in conventional cell cultures, causing elevation of the cellular O$_2$ concentration, which leads to degradation of HIF-α.”

In the present study, the cells were superfused with medium equilibrated with PO$_2$ of 130 Torr, continuously replenishing the medium in contact with the cells. As a result, when the superfusion medium was changed to one with CO, the oxygen pressure in the medium and at the cell surface did not change. Moreover, the CO-to-O$_2$ ratio was low enough to cause only partial decrease in mitochondrial respiratory capacity. Under these conditions, addition of CO would induce only a transient decrease (a few seconds) in the rate of oxygen consumption that lasted only until the energy state decreased enough to restore the respiratory rate (rate of ATP synthesis). Thus CO, by competing with oxygen for the active site of cytochrome c-oxidase and lowering the oxygen pressure “seen” by the oxidase, lowers the cellular energy state. Stabilization of HIF-1α is likely linked to the decrease in energy state.

The CB has a distinctive sensitivity to oxygen pressure, but oxygen-sensing occurs in essentially all cells and tissues. The CB and aortic bodies are notable, not as much because they sense oxygen as because they are small organs specifically designed to translate the sensed oxygen pressure into afferent neural activity. This neural activity then influences the function of other tissues in remote locations. In most cells and tissues, oxygen sensing is used to control local functions, of which a large number are oxygen dependent. Well-known examples include oxygen delivery to the tissue, including autoregulation in the brain and heart, modulation of local blood vessel density (angiogenesis), and apoptotic cell death. The oxygen pressure dependence of these tissue responses can be altered. It is known, for example, that the CB of adult animals is much more sensitive to oxygen than is CB of newborn, and this sensitivity changes continuously during the maturation process (see Refs. 8, 26). In adult animals, the oxygen sensitivity of the CB is much greater than that of the aortic body (22). These differences are consistent with the oxygen sensitivity of the tissue response not being determined just by the oxygen sensor. Rather, it indicates the sensor and sensory signal are being modulated by the metabolic environment and transmission mechanisms. The oxygen sensitivity can be, and is, modulated at several levels, including oxygen delivery to the tissue (vascular density, blood flow), the “set point” for cellular energy metabolism, and sensitivity of the neurons to the “message.” It is the “secondary” signal modulation mechanisms that determine the differences in oxygen sensitivity observed, for example, in the CB during maturation and between the aortic bodies and CBs. There is good evidence that mitochondrial oxidative phosphorylation, through cytochrome c-oxidase, is an important oxygen sensor for regulation of not only the CB and aortic body activity but also of cardiac blood flow [see Nuutinen et al. (31, 32)] and many other functions. It should be noted, however, that mitochondrial cytochrome c-oxidase is not the only oxygen-sensitive component in the cells, and not all oxygen sensing can be attributed to a single “universal” sensor. The data presented in this paper focus only on CB neural activity and HIF-1α induction in the glomus cells, showing that they share a common oxygen sensor, and this is mitochondrial cytochrome c-oxidase.

In summary, carbon monoxide (PCO$_2$ of ~350 Torr) added to normoxic medium (PO$_2$ of ~130 Torr) induced increases in sensory discharge in the isolated perfused rat CB and expression of both HIF-1α and tyrosine hydroxylase in glomus cells. The changes in afferent electrical activity were rapid (2–3 s) and fully reversed by light, the extent of reversal was much greater for monochromatic light at 430 and 590 nm than for light at 450, 550, and 610 nm. Isolated glomus cells cultured for 45 min in the presence of CO (PCO$_2$ of ~350 Torr; PO$_2$ of ~130 Torr) in the dark showed marked increase in HIF-1α. This increase was blocked by continuous illumination with white light. Monochromatic light at 430 and 590 nm also effectively blocked the increase, whereas that at 450, 550, and 610 nm did not. The changes in HIF-1α were much slower than those for the afferent electrical activity, but the similar dependencies on CO and light are consistent with their responding to the same oxygen sensor (mitochondrial cytochrome a$_3$).
GRANTS

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