Respiratory Physiology & Neurobiology 184 (2012) 158–164

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Review

NO modulation of carotid body chemoreception in health and disease☆

Esteban A. Moya[a], Julio Alcayaga[b], Rodrigo Iturriaga[a,*]

[a] Laboratorio de Neurobiología, Departamento de Fisiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile
[b] Laboratorio de Fisiología Celular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 6 February 2012
Received in revised form 29 March 2012
Accepted 29 March 2012

Keywords:
Carotid body
Nitric oxide
Intermittent hypoxia
Congestive heart failure

ABSTRACT

Nitric oxide (NO), at physiological concentrations, is a tonic inhibitory modulator of carotid body (CB) chemosensory discharges. NO modulates the chemoreception process by several mechanisms, indirectly by modifying the vascular tone and oxygen delivery, and directly through the modulation of the excitability of glomus cells and petrosal neurons. In addition to the inhibitory effect, at high concentrations NO has a dual dose-dependent effect on CB chemoreception that depends on the P_{O_2}. In hypoxic conditions, NO is primarily an inhibitory modulator of CB chemoreception, while in normoxia NO increases the chemosensory discharges. In this review, we will examine new evidence supporting the idea that NO is involved in the CB chemosensory potentiation induced by congestive heart failure (CHF) and chronic intermittent hypoxia (CIH), the main feature of obstructive sleep apnea (OSA). Evidence from patients and experimental animal models indicates that CHF and OSA, as well as CIH, potentiate the carotid hypoxic chemoreflexes, contributing to enhance the sympathetic tone. Moreover, animals exposed to CIH or to pacing-induced CHF showed enhanced baseline CB discharges in normoxia and potentiated chemosensory responses to acute hypoxia. Several molecules and pathways are altered in CHF, OSA and CIH, but the available evidence suggests that a reduced NO production in the CB plays an essential role in both diseases, contributing to enhance the CB chemosensory discharges.

1. Introduction

The carotid body (CB) plays a pivotal role in the cardiorespiratory regulation of oxygen homeostasis in mammals. Indeed, the CB is the main peripheral sensor of arterial blood P_{O_2}, P_{CO_2}, and pH levels. Hypoxia, hypercapnia and acidosis increases the frequency of the chemosensory discharges in the carotid sinus nerve, while hyperoxia, hypocapnia and alkalosis reduces the discharges (Iturriaga and Alcayaga, 2004; Iturriaga et al., 2007). It is currently accepted that the primary oxygen sensor in the CB are the chemoreceptor cells (glomus or type I cells), which are in close apposition to the nerve terminals of the chemosensory neurons, the somata of which are located in the petrosal ganglion (Iturriaga et al., 2007). In response to the natural chemosensory stimuli glomus cells depolarize, which in turn increase their intracellular [Ca^{2+}] through the entry of Ca^{+2} *via* L-type channels, and release several transmitters and modulators (Iturriaga and Alcayaga, 2004; Lopez-Barneo et al., 2001; Nurse, 2005). Several molecules have been proposed as transmitters mediating the synapses between glomus cells and petrosal neuron terminals. Among the putative transmitters, acetylcholine (ACh) and adenosine triphosphate (ATP) fulfill most of the criteria to be considered the excitatory transmitters in the CB (Iturriaga and Alcayaga, 2004; Iturriaga et al., 2007; Nurse, 2005).

In addition to the excitatory transmitters, other molecules produced within the CB modulate the chemosensory process, acting on the glomus cells, the sensory nerve endings or controlling the vascular tone and oxygen delivery (Iturriaga et al., 2007; Rey et al., 2004). The nitric oxide (NO) gas, one of the most potent vasodilators (Moncada et al., 1991) which also works as a neurotransmitter in the nervous system (Snyder, 1992), has been proposed to be an inhibitory modulator of the CB chemosensory process. Nitric oxide is enzimatically produced from the amino acid L-arginine by three different nitric oxide synthases (NOS): (i) the neuronal (nNOS), (ii) the endothelial (eNOS) and (iii) the inducible (iNOS) isoforms. The NOSs are heme proteins with an oxygenase and a reductase domain. The reductase domain conveys an electron from the NADPH to the heme group; then the NOS hydroxylates the guanidine nitrogen of the L-arginine and oxidizes the NO-hydroxy-L-arginine intermediate to NO and L-citrulline. The reaction requires two molecules of O_2. The action of NO in the biological tissues is transient, with a half-life of few seconds. The biological effects of NO are

☆ This paper is a part of special issue entitled "Gasotransmitters and Respiration", guest-edited by Prem Kumar and Chris Peers.

* Corresponding author at: Laboratorio de Neurobiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 193, Santiago, Chile.
Fax: +56 2 354 1850.
E-mail address: riturriaga@bio.puc.cl (R. Iturriaga).

1569-9048/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2012.03.019

mediated through the reaction of NO with a large number of targets such as heme groups, cysteine residues, and iron and zinc clusters (Brown, 1999; Cassina and Radi, 1996; Moncada et al., 1991).

2. Localization of nitric oxide synthases isoforms in the carotid body and autonomic neurons

Nitric oxide synthase immunoreactivity (NOS-ir) and NADPH diaphorase activities have been found in the CB and the petrosal neurons of several species (Campanucci et al., 2006; Chugh et al., 1994; Del Rio et al., 2011a; Gozal et al., 1996; Grimes et al., 1994; Hohler et al., 1994; Prabhakar et al., 1993; Tanaka and Chiba, 1994; Wang et al., 1993, 1994). Under normoxic condition, eNOS is expressed in endothelial cells of the blood vessels of the cat and rat CBs (Grimes et al., 1994; Wang et al., 1993), while the nNOS isoform is present in neuronal structures (Campanucci et al., 2006; Wang et al., 1994, 1995). The iNOS isoform is normally not found in the CB during normoxia, but is expressed in glomus cells of the rat CB following long-term sustained or intermittent hypoxic exposure (Del Rio et al., 2011a; Di Giulio et al., 2005; Ye et al., 2002). Wang et al. (1993, 1994) found the presence of NOS-ir in a plexus of C-fibers innervating the CB blood vessels and encircling the glomus cells, but not in the glomus, the sustentacular or smooth muscle cells. However, Yamamoto et al. (2006) reported the presence of positive immunoreactivity for eNOS in the rat CB glomus cells, but not for nNOS or iNOS. More recently, Del Rio et al. (2011a) confirmed that rat glomus cells express eNOS in normoxic conditions. They found eNOS-ir positive staining in the rat CB parenchyma around glomus cell clusters and in endothelial cells from capillaries and arterioles, but most of the eNOS-ir was confined to the glomus cells. Wang et al. (1995) reported that the positive NOS-ir in C-fibers associated with blood vessels was unaffected by the section of the carotid sinus nerve or the ablation of the superior cervical ganglion. On the contrary, the NOS-ir in the nerve endings encircling the glomus cells was eliminated by the section of the carotid sinus nerve. Thus, they proposed two neural sources for the NO production in the CB: parasympathetic neurons that control the vascular tone of the CB blood vessels, and the nerve endings of petrosal (sensory) neurons. Grimes et al. (1994) found that NOS-ir and NADPH diaphorase activity in the cat CB were predominantly localized in nerve fibers associated with blood vessels and only occasionally apposed to the glomus cells. They found that NOS-positive fibers originated from autonomic neurons, scantly located within and around the CB, and in the glossopharyngeal nerve. In the superior cervical ganglion, Grimes et al. (1994) found that NOS and diaphorase activities were localized in preganglionic fibers and in a small population of VIP-positive neurons, presumably cholinergic ganglion neurons. They proposed that the NOS innervation of the cat CB originated mainly from a population of dispersed parasympathetic neurons, which affect the glomus cells activity by regulating the blood vessel tone. Hohler et al. (1994) found a similar pattern of NOS-ir positive distribution in the rat CB. Most of the NOS-positive varicose nerve fibers innervate the blood vessels, and to a lesser extent encircle glomus cell clusters. Since the NOS-positive fibers persisted after carotid sinus neurotomy, they proposed that these fibers derived from intrinsic autonomic neurons. Campanucci et al. (2006) found that a small number of autonomic neurons located in the glossopharyngeal nerve and in the carotid sinus nerve expressed nNOS. Since the efferent terminals of these neurons are located near the CB glomus cells, they proposed that NO is the mediator of the efferent inhibition in the CB. This idea is supported by the observation that the electrical stimulation of the carotid sinus nerve increases the NO production in the CB, an effect prevented by the application of a specific nNOS antagonist (Valdes et al., 2003).

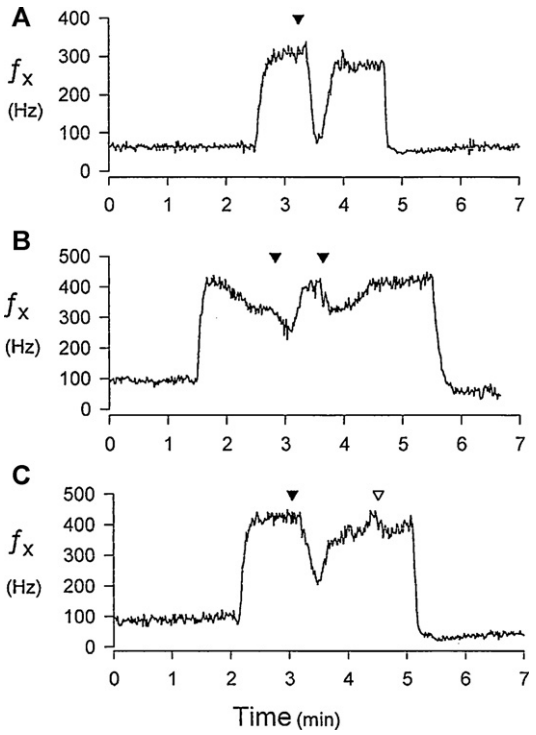

Fig. 1. Effects of injections of Tyrode equilibrated with 95% N_2 and 5% CO_2 and 25 ppm NO gas (solid arrows) on steady chemoreceptor excitation induced by hypoxia in three CBs. (A) One injection of 2 ml of Tyrode with NO. (B) Two injections of 2 ml of Tyrode with NO. (C) Comparison between one injection of 1 ml Tyrode with NO and one injection of 1 ml of Tyrode without NO (empty arrow). f_x, Frequency of chemosensory discharges.

(Reprinted from Iturriaga et al., 2000a, page 238, with permission from Elsevier).

3. Modulatory effect of NO on the carotid chemosensory process

Several studies have shown that physiological concentrations of NO exert an inhibitory tone on basal CB chemosensory discharge in normoxia, and reduce the CB chemosensory responses to hypoxia. Administration of L-arginine (Wang et al., 1994), and the NO donor molecules sodium nitroprusside (SNP), nitroglycerine and S-nitroso-N-acetylpenicillamine (SNAP) to the CB, reduces the basal discharges in normoxia and the chemosensory responses to hypoxia (Trzebski et al., 1995; Wang et al., 1993; Valdes et al., 2003). Moreover, the NO donors also reduce the responses to nicotine and NaCN in the *in vitro* cat CB (Alcayaga et al., 1997; Valdes et al., 2003). In addition, Iturriaga et al. (2000a) reported that bolus injections or the perfusion with NO-equilibrated solution at low concentration (25 ppm NO in N_2) in the perfused cat CB reduces the increased chemosensory discharges induced by hypoxia. The perfusion with NO-equilibrated solution during the hypoxic challenge reduces the rate of rise and the amplitude of the CB chemosensory response to hypoxia (Iturriaga et al., 2000a). Fig. 1 (Iturriaga et al., 2000a) shows the reduction of chemosensory activity produced by bolus injections of hypoxic Tyrode pre-equilibrated with NO gas into the perfusate line during steady chemosensory excitation induced by hypoxia in three cat CBs perfused *in vitro*. On the other hand, the inhibition of the NOS activity with N-ω-nitro-L-arginine methyl ester (L-NAME) and L-nitro-ω-arginine (L-NNA) (i) increases CB basal discharges (Fung et al., 2001; Prabhakar et al., 1993; Valdes et al., 2003; Wang

Table 1
Proposed mechanism for NO action in the CB.

Proposed mechanism	References
Control of vascular tone	Chugh et al., 1994; Wang et al., 1994; Grimes et al., 1994; Rey and Iturriaga, 2004
Inhibition of mitochondrial metabolism	Iturriaga et al., 2000b; Mosqueira and Iturriaga, 2002
Modulation of petrosal neuron excitability	Alcayaga et al., 1999; Campanucci et al., 2006
Retrograde inhibition of chemoreceptor cells	Wang et al., 1995; Campanucci et al., 2006
Modulation of ionic channels in glomus cells	Summers et al., 1999; Li et al., 2004, 2010

et al., 1994), (ii) enhances the chemosensory response induced by hypoxia in the cat CB *in vitro* (Chugh et al., 1994; Valdes et al., 2003) and *in situ* (Iturriaga et al., 1998), and (iii) enhances CB chemosensory responses to hypoxia in rats exposed to chronic hypoxia for 12 days (He et al., 2007). Thus, the experimental evidence strongly supports that NO in low concentrations is an inhibitory modulator of the CB hypoxic chemoreception. However, NO at high concentrations (1–10 μM) produced a dual effect on cat CB discharges, depending on the P_{O_2} levels. During hypoxia, NO reduces the augmented chemosensory discharges, while in normoxia NO produces chemosensory excitation (Iturriaga et al., 2000b; Mosqueira and Iturriaga, 2002). The mechanisms by which large concentrations of NO produces chemosensory excitation during normoxia remain to be determined, but the NO excitatory effect is associated with an impairment of mitochondrial electron transport and/or oxidative phosphorylation (Iturriaga et al., 2000b; Mosqueira and Iturriaga, 2002).

4. Targets and mechanisms of action of NO in the carotid body

NO modulates the oxygen chemoreception process in CB process at different sites (Table 1). Indeed, NO modulates the CB chemoreception by regulating the vascular tone, but also NO may modulate glomus cells and petrosal neurons excitability (Fig. 2). The available evidence suggests that NO exerts a tonic vasodilatation of CB vasculature, but the mechanisms underlying the NO-mediated vascular regulation are not completely understood (Rey and Iturriaga, 2004). Wang et al. (1994) found that the nonselective NOS inhibition with L-NAME resulted in a larger chemosensory excitation in the perfused cat CB than in the superfused preparation, which is devoid of vascular effects (Rey and Iturriaga, 2004). Thus, they proposed that the main inhibitory effect of NO on hypoxic CB chemoreception is mediated by vasoconstriction resulting from increased cGMP levels in smooth vascular muscle cells (Prabhakar et al., 1993). In agreement with these findings, Lahiri and Buerk (1998) found that SNAP, which reduced basal chemosensory discharges in the perfused cat CB, increased CB tissue P_{O_2} supporting the proposal that the inhibitory effect of NO on CB chemoreception is mediated by vasodilatation. Wang et al. (1995) used an *in vitro* preparation of the cat CB that allows the perfusion or superfusion of the organ. When the CB was perfused, the antidromic electrical stimulation of C-fibers in the carotid sinus nerve increased cGMP in the CB and reduced the chemosensory discharges, an effect blocked by L-NAME. However, when the CB was superfused, the stimulation of C-fibers in the carotid sinus nerve did not reduce the chemosensory discharge or increase the cGMP levels in the CB (Wang et al., 1995). Valdes et al. (2003), using an *in vitro* perfused preparation of the cat CB, compared the effects of the nonselective NOS inhibitor L-NAME and the nNOS selective inhibitor 1-(2-trifluoromethylphenyl)-imidazole (TRIM) on the chemosensory responses induced by nicotine, NaCN, and hypoxia. They found that L-NAME enhanced the chemosensory

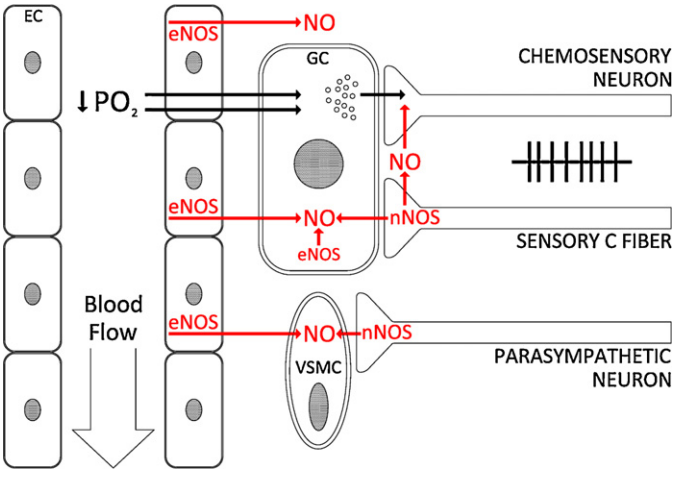

Fig. 2. Diagram of possible sources and actions for NO in the carotid body. NO effects may occur at glomus cells (GC), vascular smooth muscle cells (VSMC), endothelial cells (EC) and/or petrosal chemosensory neurons. Vascular actions of NO may regulate the P_{O_2} in the CB parenchyma by controlling the vascular tone, but it is likely that non-vascular actions of NO may modify the release of excitatory putative transmitters in glomus cells, or modulates the petrosal neuron excitability. NO produced and released from sensory C-fibers may be part of the efferent inhibition in the carotid body.

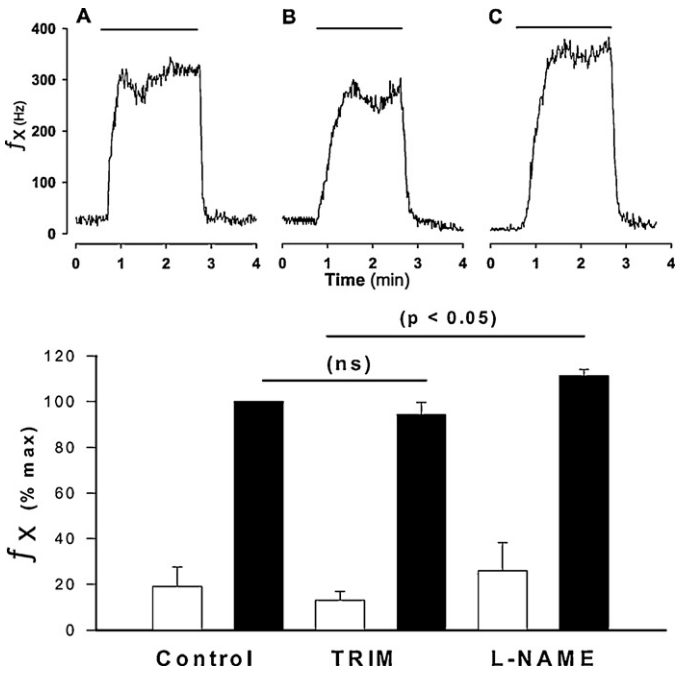

Fig. 3. Upper pannel, effect of TRIM and L-NAME on chemosensory response to hypoxia ($P_{O_2} \approx 30$ Torr). (A) control response; (B) after 10 min of perfusion with TRIM (100 μM); (C) after 10 min of perfusion with L-NAME (1 mM). Lower pannel, summary of the effects of TRIM and L-NAME on chemosensory response to hypoxia in 4 CBs during Tyrode perfusion (control) and after 10 min of perfusion with TRIM (100 μM) and after 10 min of perfusion with L-NAME (1 mM). f_X, expressed as % of maximal control response. Open bars, basal f_X; solid bars, maximal f_X. $P < 0.05$, Bonferroni test after 1-way ANOVA; ns, Not significant.
(Adapted and reprinted from Valdes et al., 2003, pages L65 and L66, with permission from the American Physiological Society).

responses to nicotine, NaCN and hypoxia, but TRIM only potentiates the chemosensory responses to high doses of NaCN. Fig. 3 (Valdes et al., 2003) shows the effects of TRIM and L-NAME on the cat CB chemosensory response to hypoxia. The chemosensory response to hypoxia was higher in the presence of L-NAME than in the presence of TRIM ($P < 0.05$). These results suggested that both eNOS and nNOS isoforms contribute to the inhibitory effect of NO in the CB, but eNOS appears to be the major source for NO in the cat CB, maintaining a tonic inhibitory effect on the chemosensory activity (Valdes et al., 2003). However, it is plausible that the NO produced by the endothelial cells may diffuse and reach the glomus cells in the CB. The proximity of the endothelial cells to the glomus cells and the nerve endings and the high baseline levels of NO (300 nM) in normoxia reported by Lahiri and Buerk (1998) in the cat CB support this interpretation.

In addition to the cGMP-mediated vascular effects, Wang et al. (1995) proposed that NO produced and released from the carotid sinus nerve C-fibers produces a retrograde inhibition of the glomus cell excitability. As mentioned previously, they used an *in vitro* preparation of the cat CB that allows the perfusion or superfusion of the organ, separating the vascular from the non-vascular effects. When the CB was perfused, the electrical stimulation of C-fibers in the carotid sinus nerve reduced the basal discharges, but when the CB was superfused the stimulation of the C-fiber stimulation failed to modify the chemosensory discharges. However, prolonged nerve stimulation (5 min) attenuated the chemosensory response to hypoxia, suggesting a direct effect of NO on glomus cells. The most plausible targets for the inhibitory effects of NO in the glomus cells are the L-type Ca^{2+} channels. Indeed, Summers et al. (1999) found that the NO donors SNP and spermine inhibit the L-type Ca^{2+} currents in rabbit glomus cells through a cGMP-independent mechanism. The inhibition of the L-type Ca^{2+} current induced by NO appears to be a direct modification of thiol groups in the Ca^{2+} channel protein, because the inhibitory NO effect was abolished by N-ethylmalemide, which prevents the NO-mediated protein nitrosylation.

The petrosal ganglion neurons are another possible target site for the inhibitory action of NO in the chemoreceptor system. Alcayaga et al. (1999) studied the modulatory effect of NO on a population of petrosal neurons projecting through the carotid sinus nerve to the cat CB, which was selectively activated by ACh applied to the isolated petrosal ganglion. They found that SNP reduced the sensitivity and amplitude of the antidromic discharges evoked by ACh, while L-NAME increased the sensitivity of the ACh-induced responses, an effect that persisted after L-NAME withdrawal. These results suggest that NO may modulate the neural activity of a population of sensory neurons of the cat petrosal ganglion, which are activated by ACh. Campanucci et al. (2006) using co-cultures of rat petrosal neurons and glomus cells found that the ATP-mediated activation of P2X receptors on petrosal neurons elicited hyperpolarization in the adjacent glomus cells. The hyperpolarization was prevented by the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide potassium (Carboxy-PTIO). They proposed that the ATP released from the glomus cells during the chemosensory excitation activate Ca^{2+}-permeable P2X receptors in the petrosal neurons, leading to Ca^{2+} influx and activation of nNOS in the nerve terminals that evokes the synthesis and release of NO, which in turn produces glomus cell hyperpolarization and decreased neurotransmitter release (Campanucci et al., 2006). Taken together, the studies of Alcayaga et al. (1999) and Campanucci et al. (2006) suggest that the chemosensory information carried by the primary sensory petrosal neurons could be locally modulated by NO.

5. Role of NO on CB chemosensory process in pathophysiological conditions

The CB chemoreceptor has been implicated in various diseases, including congestive heart failure (CHF) and obstructive sleep apnea (OSA). Evidences from patients and animal models indicate that CHF and OSA potentiate the peripheral hypoxic chemoreflexes and contribute to enhance the sympathetic tone. Moreover, studies performed in animal models provided direct evidence that exposure to chronic intermittent hypoxia (CIH), the main characteristic of OSA, or by producing pacing-induced CHF, enhance the basal CB discharges in normoxia and potentiate chemosensory responses to acute hypoxia (Del Rio et al., 2010; Iturriaga et al., 2009; Peng et al., 2003; Rey et al., 2004; Prabhakar et al., 2010; Schultz and Li, 2007).

5.1. Role of NO on the enhanced carotid chemosensory responsiveness induced by heart failure

Schultz and colleges found an enhanced CB basal discharge in normoxia and potentiated chemosensory responses to hypoxia in pacing-induced CHF rabbits (Sun et al., 1999a), which contributes to increase the sympathetic outflow in the CHF disease (Sun et al., 1999b). They also provided crucial evidence showing that angiotensin II (Ang II) and NO play a major role in the potentiation of CB chemosensory function in CHF-rabbits. Their studies provided evidence that a local activation of Ang II (Li and Schultz, 2006; Li et al., 2006) and a decreased NO production mediated by nNOS (Ding et al., 2008; Li et al., 2004, 2005, 2010; Schultz and Li, 2007) are responsible for the enhanced CB chemosensory discharges. Indeed, they found a reduced basal NO production and NADPH-diaphorase positive staining in the CB from CHF rabbits (Sun et al., 1999b; Li et al., 2005). The reduced production of NO was attributed to a decreased nNOS-ir in nerve fibers innervating the CB, and a decreased total nNOS protein expression (Li et al., 2005).

Sun et al. (1999b) found that L-NNA increased the CB basal discharges in normoxia and the chemosensory responses to hypoxia in sham rabbits, but had very little effect in the CHF rabbits. By contrast, SNAP inhibited the CB chemosensory discharges to a larger extent in the CHF than in control rabbits, suggesting that the normal tonic inhibitory effect of NO on CB chemosensory discharges is markedly attenuated in CHF rabbits. Li et al. (2005) reported that gene transfer of nNOS using an adenoviral vector (Ad.nNOS) to the CB of CHF rabbits increased the nNOS protein levels and the production of NO within the CB, and reversed the enhanced CB chemosensory discharges. In addition, they found that the specific nNOS inhibitor, S-methyl-L-thiocitrulline (SMTC) abolished the effects of Ad.nNOS on CB chemosensory discharges, but failed to increase the CB chemosensory discharges in CHF-rabbits without nNOS gene transfer, indicating a loss of the tonic inhibitory influence of NO in the CHF-animals (Li et al., 2005). These results support the idea that a down-regulation of nNOS in the CB is involved in the enhanced CB chemoreceptor discharges in CHF rabbits.

According to the current hypothesis of CB chemoreception, hypoxia depolarizes glomus cells by closing K^+ channels. Therefore, Li et al. (2004) studied the effects of NO on the outward K^+ currents (I_K) of glomus cells in CHF rabbits, and found that CHF attenuated I_K and depolarized the glomus cells. The selective Ca^{2+} dependent K^+ channel (K_{Ca}) blocker iberiotoxin reduced I_K in glomus cells from sham rabbits, but had no effect on I_K from CHF rabbits, indicating that the K_{Ca} current was already reduced in CB glomus cells from CHF rabbits. Haton and Peers (1996) and Summers et al. (1999), using conventional whole-cell patch-clamp techniques, reported that SNAP and SNP did not modify

I_K current in the rat and rabbit glomus cells. However, Li et al. (2004), using whole-cell perforated patch clamp, found that SNAP increased the I_K in glomus cells from CHF rabbits. Since SNAP had no effect, but was effective in the perforated-patch mode, Li et al. (2010) suggested that the disparity in the effects of SNAP may be due to some intracellular factor that is dialysed in conventional whole-cell recording is necessary for K^+ channel modulation by SNAP. Their results suggest that this intracellular factor is cGMP, because the effect of NO on the I_K of glomus cells was cGMP-dependent. Indeed, they found that the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ) inhibited the effect of SNAP on I_K. Thus, the effect is mainly due to the suppression of K_{Ca} channel activity elicited by decreased levels of NO, indicating that intracellular cGMP is necessary for the K_{Ca} channel modulation by NO.

More recently, Li et al. (2010) studied the effects of nNOS transgene on the K^+ currents in CB glomus cells from CHF rabbits. Using single-cell real-time RT-PCR and immunofluorescent techniques, they found that nNOS mRNA and protein are expressed in the rabbit CB glomus cells and that CHF decreases the expression of nNOS both at mRNA and protein levels in CB glomus cells. The adenoviral nNOS transfection increases the nNOS innmunofluorecence and partially increases the attenuated K^+ currents in glomus cells from CHF rabbits. The NO donor SMTC and the BK channel blocker iberiotoxin suppress the K^+ currents in the glomus cells and fully abolish the effect of Ad.nNOS on the K^+ currents in CHF cells. These results suggest that endogenous nNOS is involved in the regulation of BK channels in rabbit CB glomus cells and, importantly, a reduced nNOS expression mediates the suppression of BK currents in glomus cells from CHF rabbits.

5.2. Role of NO on the enhanced carotid chemosensory responsiveness induced by chronic intermittent hypoxia

The enhanced CB chemosensory response to hypoxia induced by CIH has been attributed to oxidative stress (Del Rio et al., 2010, 2011b; Iturriaga et al., 2009; Peng et al., 2003; Prabhakar et al., 2010), which increases the expression of pro-inflammatory cytokines and ET-1in the CB (Del Rio et al., 2011a). However, less is known about the role played by the NOS isoforms and NO in the enhanced CB chemosensory responses to hypoxia induced by CIH. Recently, we studied the expression of TNF-α, IL-1β, ET-1, iNOS and eNOS and 3-nitrotirosine (3-NT) in the CB, along with the progression of potentiated CB chemosensory responses to hypoxia in rats exposed to CIH (5%O_2, 12 times/h per 8 h) for 7–21 days (Del Rio et al., 2011a). Exposure to CIH for 7 days enhanced CB chemosensory responses to hypoxia and the expression of 3-NT, effects that persisted until day 21 of CIH exposure. In addition, CIH produced a transient 2-fold increase of ET-1 at 7 days, a decrease in eNOS immunoreactivity, and a delayed, but progressive local increase of TNF-α, IL-1β and iNOS, which was not associated with changes in systemic plasma levels or immune cell invasion of the CB. Fig. 5 (Del Rio et al., 2011a) shows the effects of CIH on ET-1, eNOS and iNOS-immunoreactivity in the CB tissue from rats exposed to 7, 14 and 21 days of CIH. ET-1-ir was found in Sham and CIH-treated CBs. Positive ET-1-ir signal was present in perilobular areas, which contain capillaries and nerve fibers encircling the glomus cell clusters (glomoids), and in glomus cells defined by the ovoid-like morphology and prominent nuclei. Our results suggest that the expression of chemosensory modulators such as NO and ET-1, and pro-inflammatory cytokines in the CB may have a different temporal contribution to the CB chemosensory potentiation induced by CIH. In addition to the transient increase in ET-1, our results showed a significant decrease in the eNOS in the CB at 7 days of CIH, suggesting that CIH may decrease the NO levels in the CB. Accordingly, we measured the NO production -*via* nitrite

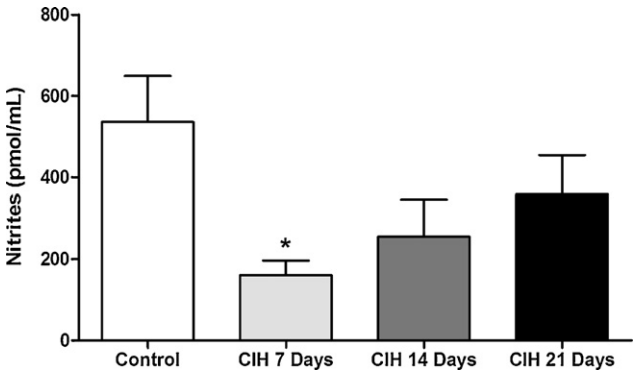

Fig. 4. Effect of intermittent hypoxia on CB NO production. NO was measured by the generation of nitrite in the incubation medium of CBs from control rats ($n = 7$), and from rats exposed to 7 ($n = 6$), 14 ($n = 4$) and 21 days ($n = 7$) of CIH (Del Rio et al., 2010). *$p < 0.05$, Newman–Keuls after one way ANOVA. CBs were surgically removed from 200 g male rats and collected in cold modifided Tyrode's buffer solution equilibrated with 100% O_2, pH 7.40. The CBs were incubated in 100 μl Tyrode's solution equilibrated with 20% O_2 and 5% CO_2 for 5 min at 37 ± 0.5 °C. NO was measured using a chemoluminiscence Sievers 280 NO analyzer to detect the NO produced by the reduction of nitrites to NO (Boric et al., 1999).

generation in the incubation medium (Boric et al., 1999)-, from rat CBs after 7, 14 or 21 days of CIH. We found a reduction in the NO production after 7 days of CIH exposure that correlates with the reduced eNOS expression (Fig. 4). Since NO at low concentration is considered an inhibitory modulator of CB chemosensory discharges (Iturriaga et al., 2000a; Rey and Iturriaga, 2004), a reduced NO level may contribute to enhance the basal CB discharges and chemosensory responses to hypoxia. This interpretation is supported by the finding of Marcus et al. (2010), showing that CIH decreased the expression of the nNOS in the rat CB, suggesting that the removal of the normal inhibitory NO influence contributes to enhancing the CB chemosensory responses to hypoxia.

In addition to the reduced expression of eNOS, our results showed that iNOS-ir increased at 21 days of CIH exposure. Since iNOS produces higher amounts of NO, it is likely that the NO production will increase in the CB during long-term CIH. As mentioned before, high NO concentration increases carotid chemosensory discharges. Indeed, Iturriaga et al. (2000b) measured the cat CB chemosensory responses to hypoxia and NO with NO-selective carbon-fiber microelectrodes inserted into the CB. Application of the NO donors SNAP and NOC-9 transiently reduced the increased hypoxic chemosensory discharges in a dose-dependent manner. However, during normoxia injections of NO donors increased the chemosensory discharges, showing a dual effect of NO on carotid chemoreception depending on P_{O_2} levels. Therefore, we proposed that high NO levels or its metabolite peroxynitrite may account for the increased chemosensory discharge, because high NO and peroxynitrite inhibit the electron transport chain and oxidative phosphorylation (Brown, 1999; Cassina and Radi, 1996). We found a marked increased of 3-NT-ir in the CB from rats exposed to CIH for 7–21 days, which correlates with the enhanced chemosensory responses to hypoxia (Del Rio et al., 2011a), supporting the idea that oxidative/nitrosative stress plays a critical role in CB chemosensory potentiation in CIH (Del Rio et al., 2010; Iturriaga et al., 2009; Prabhakar et al., 2010). The increase in 3-NT-ir found in the CB from rats exposed for 7–21 days to CIH, suggests that peroxynitrite formation due to the reaction of NO with the superoxide radical is a critical step in the CB chemosensory potentiation induced by CIH (Del Rio et al., 2010, 2011a). Moreover, ascorbic acid, which prevented the CB potentiation induced by CIH, also reduced the 3-NT formation in the CB (Del Rio et al., 2010). Peroxynitrite modifies tyrosine and tryptophane residues, iron sulfur clusters, zinc thiolates and other residues, impairing DNA, lipids and

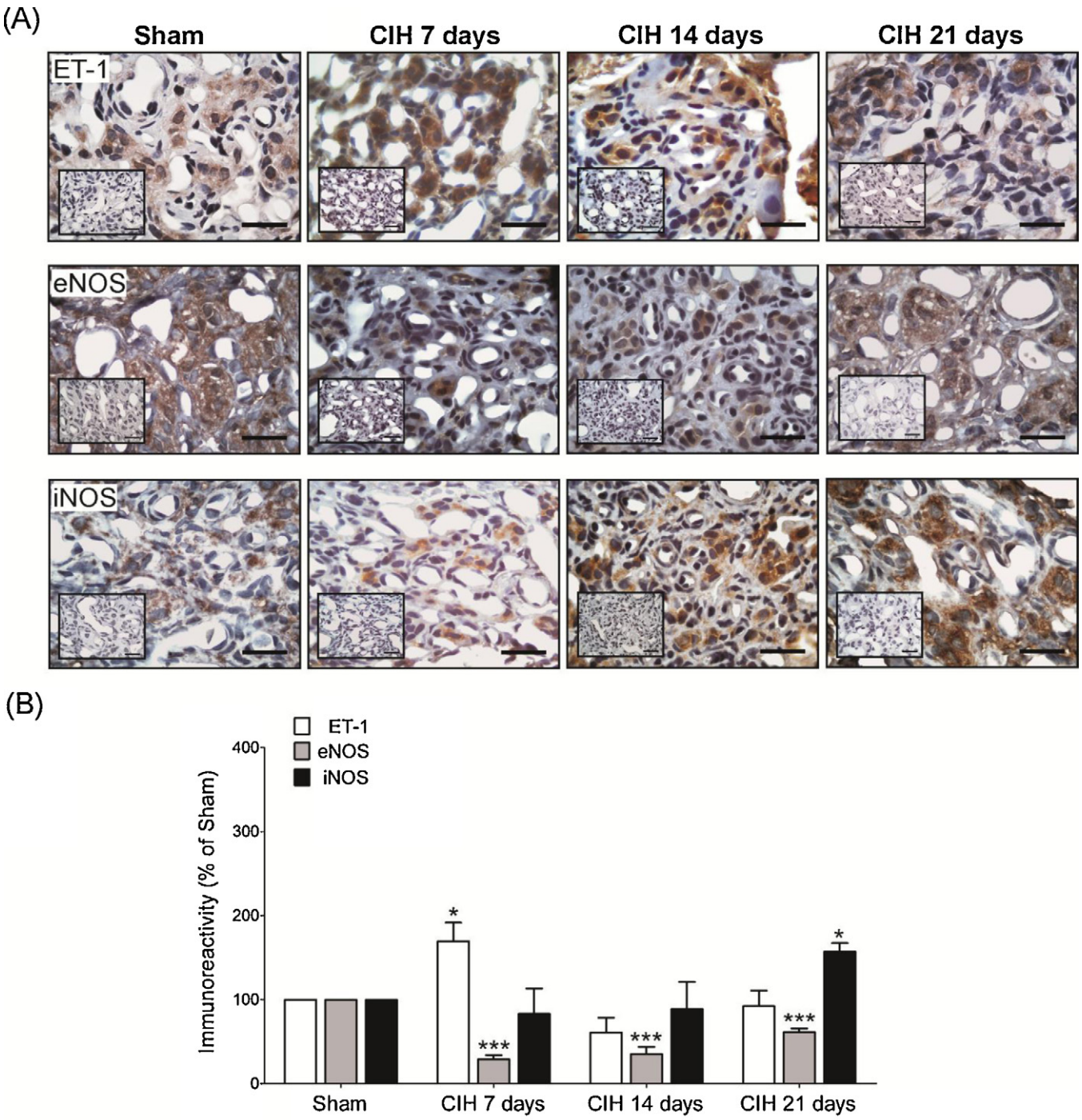

Fig. 5. Time-course of the effects of CIH on ET-1, eNOS and iNOS in the rat CB. (A) Positive immunoreactivity for ET-1, eNOS and iNOS in the CB from a Sham rat and rats exposed to 7, 14 and 21 days of CIH. Sections were counterstained with Harris-Hematoxylin. Inset, negative control by omission of the primary antibody. Scale bar, 20 μm. B, Quantification of the effects of CIH on the expression ET-1, eNOS and iNOS. ***$P < 0.001$; *$P < 0.05$ compared to sham condition. Newman–Keuls test after one-way ANOVA. $n = 6–8$.

(Reprinted from Del Rio et al., 2011a, page 78, with permission from Elsevier)

proteins (Ferrer-Sueta and Radi, 2009). Further studies are required to determine the role played by NO in the potentiation of the CB chemosensory in animals exposed to CIH.

6. Conclusions

The experimental evidence supports that NO at physiological concentration is an inhibitory modulator of the CB chemosensory activity. NO modulates the chemosensory process by controlling the vascular tone within the CB, but also it may modify the excitability of glomus cells and petrosal neurons. New evidence suggest that a reduced NO production is involved in the potentiation of CH chemosensory basal discharges and chemosensory responses to hypoxia induced by chronic intermittent hypoxia and heart failure. Nevertheless, more studies are necessary to understand the participation of NO in the pathological process, because the NO-induced alterations in CB function are important for the systemic consequences of chronic diseases.

Acknowledgements

This work was supported by grants 1100405 (RI) and 1090157 (JA) from the National Fund for Scientific and Technological Development of Chile (FONDECYT). Esteban A. Moya was supported by a CONICYT AT-24110172 fellowship.

References

Alcayaga, J., Barrios, M., Bustos, F., Miranda, G., Molina, V., Iturriaga, R., 1999. Modulatory effect of nitric oxide on acetylcholine-induced activation of cat petrosal ganglion neurons *in vitro*. Brain Research 825, 194–198.

Alcayaga, J., Iturriaga, R., Ramirez, J., Readi, R., Quezada, C., Salinas, P., 1997. Cat carotid body chemosensory response to non-hypoxic stimuli are inhibited by sodium nitroprusside both *in situ* and *in vitro*. Brain Research 767, 384–438.

Boric, M.P., Figueroa, X.F., Donoso, M.V., Paredes, A., Poblete, I., Huidobro-Toro, J.P., 1999. Rise in endothelium-derived NO after stimulation of rat perivascular sympathetic mesenteric nerves. American Journal of Physiology 277, H1027–H1035.

Brown, C.G., 1999. Nitric Oxide and mitochondrial respiration. Biochimica Et Biophysica Acta 1411, 351–369.

Campanucci, V.A., Zhang, M., Vollmer, C., Nurse, C.A., 2006. Expression of multiple P2X receptors by glossopharygeal neurons projecting to rat carotid body O_2-chemoreceptors: role in nitric oxide-mediated efferent inhibition. Journal of Neuroscience 26, 9482–9493.

Cassina, A., Radi, R., 1996. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Archives of Biochemistry and Biophysics 328, 309–316.

Chugh, D.K., Katayama, M., Mokashi, A., Bebout, D.E., Ray, D.K., Lahiri, S., 1994. Nitric oxide-related inhibition of carotid chemosensory nerve activity in the cat. Respiration Physiology 97, 147–156.

Del Rio, R., Moya, E.A., Iturriaga, R., 2010. Carotid body and cardiorespiratory alterations in intermittent hypoxia: the oxidative link. European Respiratory Journal 36, 143–150.

Del Rio, R., Moya, E.A., Iturriaga, R., 2011a. Differential expression of pro-inflammatory cytokines, endothelin-1 and nitric oxide synthases in the rat carotid body exposed to intermittent hypoxia. Brain Research 1395, 74–85.

Del Rio, R., Muñoz, C., Arias, P., Court, F.A., Moya, E.A., Iturriaga, R., 2011b. Chronic intermittent hypoxia-induced vascular enlargement and VEGF upregulation in the rat carotid body is not prevented by antioxidant treatment. American Journal of Physiology. Lungcellular and Molecular Physiology 301, L702–L711.

Di Giulio, C., Bianchi, G., Cacchio, M., Artese, L., Rapino, C., Macri, M.A., Di Ilio, C., 2005. Oxygen and life span: chronic hypoxia as a model for studying HIF-1alpha, VEGF and NOS during aging. Respiratory Physiology & Neurobiology 147, 31–38.

Ding, Y., Li, Y.L., Schultz, H.D., 2008. Downregulation of carbon monoxide as well as nitric oxide contributes to pheripheral chemoreflex hypersensitivity in heart failure rabbits. Journal of Applied Physiology 105, 14–23.

Ferrer-Sueta, G., Radi, R., 2009. Chemical biology of peroxynitrite: kinetics, diffusion and radicals. ACS Chemical Biology 4, 161–177.

Fung, M.L., Ye, J.S., Fung, P.C., 2001. Acute hypoxia elevates nitric oxide generation in rat carotid body in vitro. Pflugers Archive 442, 903–909.

Gozal, D., Gozal, E., Gozal, Y.M., Torres, J.E., 1996. Nitric oxide synthase isoforms and peripheral chemoreceptor stimulation in conscious rats. Neuroreport 7, 1145–1148.

Grimes, P.A., Lahiri, S., Stone, R., Mokashi, A., Chugh, D., 1994. Nitric oxide synthase occurs in neurons and nerve fibers of the carotid body. Advances in Experimental Medicine and Biology 360, 221–224.

Haton, C.J., Peers, C., 1996. Hypoxic inhibition of K^+ currents in isolated rat type I carotid body cells: evidence against the involvement of cyclic nucleotides. Pflügers Archive 433, 129–135.

He, L., Chen, J., Liu, X., Dinger, B., Fidone, S., 2007. Enhanced nitric oxide-mediated chemoreceptor inhibition and altered cyclic GMP signaling in rat carotid body following chronic hypoxia. American Journal of Physiology. Lungcellular and Molecular Physiology 293, L1463–L1468.

Hohler, B., Mayer, B., Kummer, W., 1994. Nitric oxide synthase in the rat carotid body and carotid sinus. Cell and Tissue Research 6, 559–564.

Iturriaga, R., Alcayaga, J., Rey, S., 1998. Sodium nitroprusside blocks the cat carotid chemosensory inhibition induced by dopamine, but not that by hyperoxia. Brain Research 799, 26–34.

Iturriaga, R., Mosqueira, M., Villanueva, S., 2000a. Effects of nitric oxide gas on carotid body chemosensory response to hypoxia. Brain Research 855, 282–286.

Iturriaga, R., Villanueva, S., Mosqueira, M., 2000b. Dual effects of nitric oxide on carotid body chemoreception. Journal of Applied Physiology 89, 1005–1012.

Iturriaga, R., Alcayaga, J., 2004. Neurotransmission in the carotid body. Transmitters and modulators between glomus cells and petrosal ganglion nerve terminals. Brain Research Reviews 47, 46–53.

Iturriaga, R., Varas, R., Alcayaga, J., 2007. Electrical and pharmacological properties of petrosal ganglion neurons that innervate the carotid body. Respiratory Physiology & Neurobiology 157, 130–139.

Iturriaga, R., Moya, E.A., Del Rio, R., 2009. Carotid body potentiation induced by intermittent hypoxia: implications for cardiorespiratory changes induced by sleep apnoea. Clinical and Experimental Pharmacology and Physiology 36, 1197–1204.

Lahiri, S., Buerk, D.G., 1998. Vascular and metabolic effects of nitric oxide synthase inhibition evaluated by tissue P_{O_2} measurements in carotid body. In: Hudetz, A.G., Bruley, D.F. (Eds.), Oxygen Transport to Tissue XX. Plenum Press, New York, pp. 455–460.

Li, Y.L., Sun, S.Y., Overholt, J.L., Prabhakar, N.R., Rozanski, G.J., Zucker, I.H., Schultz, H.D., 2004. Attenuated outward potassium currents in carotid body glomus cells of heart failure rabbit: involvement of nitric oxide. Journal of Physiology 555, 219–229.

Li, Y.L., Li, Y.F., Liu, D., Cornish, K.G., Patel, K.P., Zucker, I.H., Channon, K.M., Schultz, H.D., 2005. Gene transfer of neuronal nitric oxide synthase to carotid body reverses enhanced chemoreceptor function in heart failure rabbits. Circulation Research 97, 260–267.

Li, Y.L., Schultz, H.D., 2006. Enhanced sensitivity of Kv channels to hypoxia in the rabbit carotid body in heart failure: role of angiotensin II. Journal of Physiology 575, 215–227.

Li, Y.L., Xia, X.H., Zheng, H., Gao, L., Li, Y.F., Liu, D., Patel, K.P., Wang, W., Schultz, H.D., 2006. Angiotensin II enhances carotid body chemoreflex control of sympathetic outflow in chronic heart failure rabbits. Cardiovascular Research 71, 129–138.

Li, Y.L., Zheng, H., Ding, Y., Schultz, H.D., 2010. Expression of neuronal nitric oxide synthase in rabbit carotid body glomus cells regulates large-conductance Ca^{2+} activated potassium currents. Journal of Neurophysiology 103, 3027–3033.

Lopez-Barneo, J., Pardal, R., Ortega-Saenz, P., 2001. Cellular mechanism of oxygen sensing. Annual Review of Physiology 63, 259–287.

Marcus, N.J., Li, Y.L., Bird, C.E., Schultz, H.D., Morgan, B.J., 2010. Chronic intermittent hypoxia augments chemoreflex control of sympathetic activity: role of the angiotensin II type 1 receptor. Respiratory Physiology & Neurobiology 171, 36–45.

Moncada, S., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews 43, 109–141.

Mosqueira, M., Iturriaga, R., 2002. Carotid body chemosensory excitation induced by nitric oxide: involvement of mitochondrial metabolism. Respiratory Physiology & Neurobiology 131, 175–187.

Nurse, C.A., 2005. Neurotransmission and neuromodulation in the chemosensory carotid body. Autonomic Neuroscience 120, 1–9.

Peng, Y.J., Overholt, J.L., Kline, D., Kumar, G.K., Prabhakar, N.R., 2003. Induction of sensory long-term facilitation in the carotid body by intermittent hypoxia: implications for recurrent apneas. Proceedings of the National Academy of Sciences of the United States of America 100, 10073–10078.

Prabhakar, N.R., Kumar, C.K., Chang, C.H., Agani, F.H., Haxhiu, M.A., 1993. Nitric oxide in the sensory function of the carotid body. Brain Research 625, 16–22.

Prabhakar, N.R., Kumar, G.K., Nanduri, J., 2010. Intermittent hypoxia augments acute hypoxic sensing via HIF-mediated ROS. Respiratory Physiology & Neurobiology 174, 230–234.

Rey, S., Del Rio, R., Alcayaga, J., Iturriaga, R., 2004. Chronic intermittent hypoxia enhances cat chemosensory and ventilatory responses to hypoxia. Journal of Physiology 560, 577–586.

Rey, S., Iturriaga, R., 2004. Endothelins and nitric oxide: vasoactive modulators of carotid body chemoreception. Current Neurovascular Research 1, 463–465.

Schultz, H.D., Li, Y.L., 2007. Carotid body function in heart failure. Respiratory Physiology & Neurobiology 157, 171–185.

Sun, S.Y., Wang, W., Zucker, I.H., Schultz, H.D., 1999a. Enhanced peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure. Journal of Applied Physiology 86, 1264–1272.

Sun, S.Y., Wang, W., Zucker, I.H., Schultz, H.D., 1999b. Enhanced activity of carotid body chemoreceptors in rabbits with heart failure: role of nitric oxide. Journal of Applied Physiology 86, 1273–1282.

Summers, B.A., Overholt, J.L., Prabhakar, N.R., 1999. Nitric oxide inhibits L-type Ca^{2+} current in glomus cells of the rabbit carotid body via a cGMP-independent mechanism. Journal of Neurophysiology 81, 1449–1457.

Snyder, S.H., 1992. Nitric oxide: first in a new class of neurotrasnmitters. Science 257, 494–496.

Tanaka, K., Chiba, T., 1994. Nitric oxide synthase containing neurons in the carotid body and sinus of the guinea pig. Microscopy Research and Technique 29, 90–93.

Trzebski, A., Sato, Y., Susuki, A., Sato, A., 1995. Inhibition of nitric oxide synthesis potentiates the responsiveness of carotid chemoreceptors to systemic hypoxia in the rat. Neuroscience Letters 190, 29–33.

Valdes, V., Mosqueira, M., Rey, S., Del Rio, R., Iturriaga, R., 2003. Inhibitory effects of NO on carotid body: contribution of neural and endothelial nitric oxide synthase isoforms. American Journal of Physiology. Lungcellular and Molecular Physiology 284, 57–68.

Wang, Z.Z., Bredt, D.S., Fidone, S.J., Stensaas, L.J., 1993. Neurons synthesizing nitric oxide innervate the mammalian carotid body. Journal of Comparative Neurology 336, 419–432.

Wang, Z.Z., Stensaas, L.J., Bredt, D.S., Dinger, B.G., Fidone, S.D., 1994. Localization and actions of nitric oxide in the cat carotid body. Neuroscience 60, 275–286.

Wang, Z.Z., Stensaas, L.J., Dinger, B.G., Fidone, S.J., 1995. Nitric oxide mediates chemoreceptor inhibition in the cat carotid body. Neuroscience 65, 217–229.

Yamamoto, Y., König, P., Henrich, M., Dedio, J., Kummer, W., 2006. Hypoxia induces production of nitric oxide and reactive oxygen species in glomus cells of rat carotid body. Cell and Tissue Research 325, 3–11.

Ye, J.S., Tipoe, G.L., Fung, P.C., Fung, M.L., 2002. Augmentation of hypoxia-induced nitric oxide generation in the rat carotid body adaptated to chronic hypoxia: an involvement of constitutive and inducible nitric oxide synthases. Pflugers Archives 444, 178–185.