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REVIEW

Autonomic innervation of the carotid body as a determinant of its sensitivity: implications for cardiovascular physiology and pathology

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Abstract

The motivation for this review comes from the emerging complexity of the autonomic innervation of the carotid body (CB) and its putative role in regulating chemoreceptor sensitivity. With the carotid bodies as a potential therapeutic target for numerous cardiorespiratory and metabolic diseases, an understanding of the neural control of its circulation is most relevant. Since nerve fibres track blood vessels and receive autonomic innervation, we initiate our review by describing the origins of arterial feed to the CB and its unique vascular architecture and blood flow. Arterial feed(s) vary amongst species and, unequivocally, the arterial blood supply is relatively high to this organ. The vasculature appears to form separate circuits inside the CB with one having arterial venous anastomoses. Both sympathetic and parasympathetic nerves are present with postganglionic neurons located within the CB or close to it in the form of paraganglia. Their role in arterial vascular resistance control is described as is how CB blood flow relates to carotid sinus afferent activity. We discuss non-vascular targets of autonomic nerves, their possible role in controlling glomus cell activity, and how certain transmitters may relate to function. We propose that the autonomic nerves sub-serving the CB provide a rapid mechanism to tune the gain of peripheral chemoreflex sensitivity based on alterations in blood flow and oxygen delivery, and might provide future therapeutic targets. However, there remain a number of unknowns regarding these mechanisms that require further research that is discussed.

Keywords Carotid body • Glomus cell • Vasculature • Autonomic innervation • Chemoreflex sensitivity

1. Introduction

The carotid body (CB) has been highlighted as a potential therapeutic target for the treatment of cardiovascular diseases, including hypertension, heart failure, and breathing disturbances.[1–7] Recent studies have suggested that in some cardiovascular disorders, the carotid bodies generate aberrant hyperreflexia and tonicity. Via reflex circuitry this tone powers the generation of excessive sympathetic activity, which is associated with the development and maintenance of disease, and contributes to end-organ damage.[2,5,7,8] The mechanisms by which hyperreflexia and aberrant tone are generated are not fully understood but include: up-regulated expression of P2X3 purinoceptor,[2] reduced carbon monoxide levels,[9,10] high levels of hydrogen sulfide and reactive oxygen species,[9,10] up-regulated proinflammatory cytokines (inflammatory mediators), and cytokine receptors in the CB.[11] There may also be changes in the expression of ion channels or activity of intracellular calcium and second messenger systems within glomus cells that regulate their excitability.[12–14]

Experimentally, one way to ascertain the role of carotid bodies in cardiorespiratory disease is to denervate or remove them. These manoeuvres reduce arterial pressure and sympathetic tone in spontaneously hypertensive rats and hypertensive patients.[15–17] In addition, in animals with heart failure, removal of carotid bodies improved autonomic imbalance and cardiac pump function as well as resolving the breathing disturbances.[18,19] In chronic heart failure, it has been proposed that the reduced cardiac output decreases blood supply to the CB that causes

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their sensitization and tonicity.[7,20,21] In addition, hypoperfusion of the CB in heart failure may be related to changes in the activity of a mechano-activated transcription factor, called Kruppel-like factor 2.[7,21,22] Studies in humans and rats have highlighted the regulatory role of the CB for glucose homeostasis. Indeed, hypoglycaemia has been shown to: (i) increase ventilation[23–27] and (ii) increase CO_2 sensitivity through adrenaline release.[28,29] Together, these effects may avert acidosis in diseases like diabetes.

Removal of carotid bodies in patients with cardiovascular and respiratory disease, as a therapeutic approach, may have deleterious consequences. This is based on the facts that many patients have comorbidities and that the CB is a multi-modal receptor with at least 20 known physiological functions including: respiratory,[30,31] cardiac/vascular,[32,33] neurohumoral regulation,[25,34,35] and behavioural.[6] Thus, its removal will likely disrupt numerous homeostatic functions thereby increasing susceptibility to side-effects. This outcome was exemplified in a recent study where nocturnal oxygen desaturations worsened in patients with heart failure and sleep apnoea.[36] Thus, understanding the changes that occur within the CB in pathological states and the emerging mechanisms underpinning aberrant hyperreflexia and tonicity become essential if one is to attempt to pharmacologically antagonize these pathological conditions in humans. Given that, the CB is sensitive to blood flow and that reductions in cardiac output appear to trigger sensitization in heart failure,[20] we have considered the mechanisms controlling blood flow to the CB and whether this knowledge might provide novel targets that allow therapeutic manipulation of its sensitivity and tonicity.

Between the 1930s and 1980s, studies on CB vascularization and its autonomic innervation were described in numerous non-rodent species. Since then, few studies have been performed confirming these findings or adding additional information about the functional control of the circulation within the CB. This may reflect the relatively small size of the CB when transitioning from dogs and cats to rats and mice; notably, there is a dearth of data in the mouse. Therefore, the aim of the current review is to understand the role of the autonomic innervation of the CB and how it might affect its sensitivity through. We survey much of the existing historical literature as well as more recent findings about the CB vasculature and its neural innervation emphasizing its neurohumoral control in different species, including human in health and disease conditions.

2. The origin of arterial blood supply to the CB in different species

The CB is considered to be one of the most densely vascularized organs in the body.[37] The origin of the arterial blood supply to the CB varies between species, and within the same species (*Table 1* and *Figure 1*). The pioneering study from Schaper[38] did not find a common arterial blood supply to the CB in human, cat, sheep, rabbit, and calf. He described that the CB in humans received its supply from a small artery with its origin at the bifurcation of the common carotid artery.[38] In contrast, in other species (cat, sheep, rabbit, and calf) he described that the arterial feed originated from several distinct branches from the carotid arteries (see below for details). In the next sections, the CB blood supply will be described for each species.

2.1 Human

In humans, the origin of CB blood supply was found to come from a small artery called the 'glomic artery' which was found to have numerous origins across individuals (*Table 1* and *Figure 1A*). These included the bifurcation of the common carotid artery,[39] the external carotid, internal carotid, ascending pharyngeal or vertebral arteries, or even the thyrocervical trunk—a branch of the subclavian artery.[40–42] Studies have shown that the origin of these arteries may be associated with ethnicity.[42,44] Muthoka *et al.*[42] compared the source of arterial blood supply to the CB in Kenyan vs. British cohorts and observed that the arterial blood supply was sourced from the carotid bifurcation in 88% of the British and 51.4% of the Kenyan population. In addition, while arterial origins from ascending pharyngeal, external, and internal carotid arteries made up only 12% of the cases in the British population, almost 50% of the Kenyan's demonstrated these feeds.[39,42] Thus, it is clear that there is a huge variety in the origin of the CB blood supply in humans, and this difference might be related to ethnicity. However, to the best of our knowledge, it is unknown whether this anatomical divergence would be associated with different physiological and/or pathophysiological functions.

2.2 Monkey

In monkeys (*Macaca fascicularis*), the CB is situated between the occipital artery and the medial portion of the internal carotid artery.[46] The morphometric study of the CB from two female cynomologus monkeys conducted by Hansen[46] did not describe where the blood supply of the CB originated. However, considering the position of the CB in the monkey, it is reasoned that the blood supply originates from one or more of the following arteries: occipital, external carotid, and/or internal carotid (*Table 1* and *Figure 1B*).

2.3 Sheep and goat

In these species, glomus tissue is not found in a single compact 'body' but scattered along the main arteries in the neck.[48] However, main clumps of glomus tissue are supplied by a branch of the occipital artery[48,49] as the internal carotid artery is small, becoming non-patent in these species (*Table 1* and *Figure 1C*).[64]

2.4 Dog, cat, and rabbit

The blood supply of the canine CB originates from a small artery branching off either the proximal part of the occipital artery or the external carotid artery[50,65,66] or ascending pharyngeal artery (*Table 1* and *Figure 1D*).[50] In cats, Davis and Story[51] indicated that its blood supply originated from the root of either the occipital or pharyngeal arteries; this was confirmed by Chungcharoen *et al.*[50] and Seidl[52] who also described the external carotid artery as an additional possibility (*Table 1* and *Figure 1E*). In the rabbit, Chungcharoen *et al.*[50] found the CB feeder artery arose from either the external or internal carotid arteries, or from the bifurcation of the common carotid artery (*Table 1* and *Figure 1F*).

2.5 Guinea pig

Kondo[67] revealed that the CB of guinea pig was located close to the origin of ascending pharyngeal artery, but its blood supply was not described. Despite this, it is probable that the blood supply to the CB comes from the ascending pharyngeal artery in guinea pig (*Table 1* and *Figure 1G*). This species does not have internal carotid arteries.[67]

2.6 Rat

In the rat, McDonald and Larue[58] described that the blood supply to the CB arises from a single artery called the 'CB artery', which originates

Table 1 Possible origins of carotid body blood supply in different species

Species	Origin	Carotid body: size/weight	Reference (s)
Human	Carotid bifurcation External carotid artery Internal carotid artery Ascending pharyngeal artery Vertebral artery Thyrocervical trunk	1.5–7 mm/12–18 mg	Heath *et al.*[39] Sarrat-Torres *et al.*[40] Ozay *et al.*[41] Muthoka *et al.*[42] Heath *et al.*[43] Khan *et al.*[44] Nguyen *et al.*[45]
Monkey	Not known	0.8–1.3 mm/not known	Hansen[46] Clarke *et al.*[47]
Sheep and goat	Occipital artery	1.1 mm/10 mg	Sadik *et al.*[48] Najafi *et al.*[49]
Dog	Occipital artery Ascending pharyngeal artery External carotid artery A muscle branch of the external carotid artery	1–3 mm/not known	Chungcharoen *et al.*[50]
Cat	Occipito-ascending pharyngeal trunk Occipital artery Ascending pharyngeal artery External carotid artery	0.45–1.2 mm/2 mg	Davis and Story[51] Chungcharoen *et al.*[50] Seidl[52] Clarke *et al.*[53] Jones[54]
Rabbit	External carotid artery Internal carotid artery or carotid bifurcation	0.8–1.9 mm/not known	Chungcharoen *et al.*[50] Clarke and de Burgh Daly[55] Eken *et al.*[56]
Guinea pig	Not known	0.5 mm/0.08 mg	Clarke and de Burgh Daly[55] Docio *et al.*[57]
Rat	External carotid artery Occipital artery	0.4–0.8 mm/0.06 mg	McDonald and Larue[58] Habeck *et al.*[59] Unur and Aycan[60] Hess[61] Clarke and de Burgh Daly[55] Clarke *et al.*[62] McDonald[63]
Mouse	Not known	0.4 mm/not known	Clarke and de Burgh Daly[55]

from either the external carotid artery or the occipital artery (*Table 1* and *Figure 1H*); this was confirmed subsequently.[59,60]

2.7 Mouse

To the best of our knowledge, the vascularization of the mouse CB has not been fully described. However, since the CB of mouse is located very close to the superior cervical ganglion (SCG) lying between external and internal carotid arteries,[68,69] we suggest its blood supply comes from either the external carotid artery or occipital artery as described in rats (*Table 1* and *Figure 1I*

3. Specializations of the intra-CB vascularization and microvascularization

Of all species, vascularization of the CB was studied comprehensively in the rat. Based on this and the density of current studies on CB carried out in the rat, our discussion in this specific section, will be based exclusively on this species.

McDonald and Larue[58] thoroughly described the ultrastructure and connections of blood vessels of the rat CB. According to their work, the CB artery enters the CB, immediately divides into three or four first-order branches, and then, into at least five second-order branches.[58] Of these secondary branches, about three branches—which are composed of continuous non-fenestrated endothelium with one or two layers of smooth muscle and a thin adventitial layer—supply the CB while the others go to adjacent structures, such as the carotid sinus nerve (CSN), vagus nerve, SCG, and nodose ganglion.[58] The arterioles supplying the glomus cells originate from the third- and fourth-order branches of the CB artery, have a diameter <15 μm, and at their distal end generate a capillary network.[58,70] Clarke *et al.*,[47] described the small vessels (i.e. capillaries) comprised ~50–60% of all vascular component of the CB. In Long-Evans rats, the microvascularization of the CB is composed of

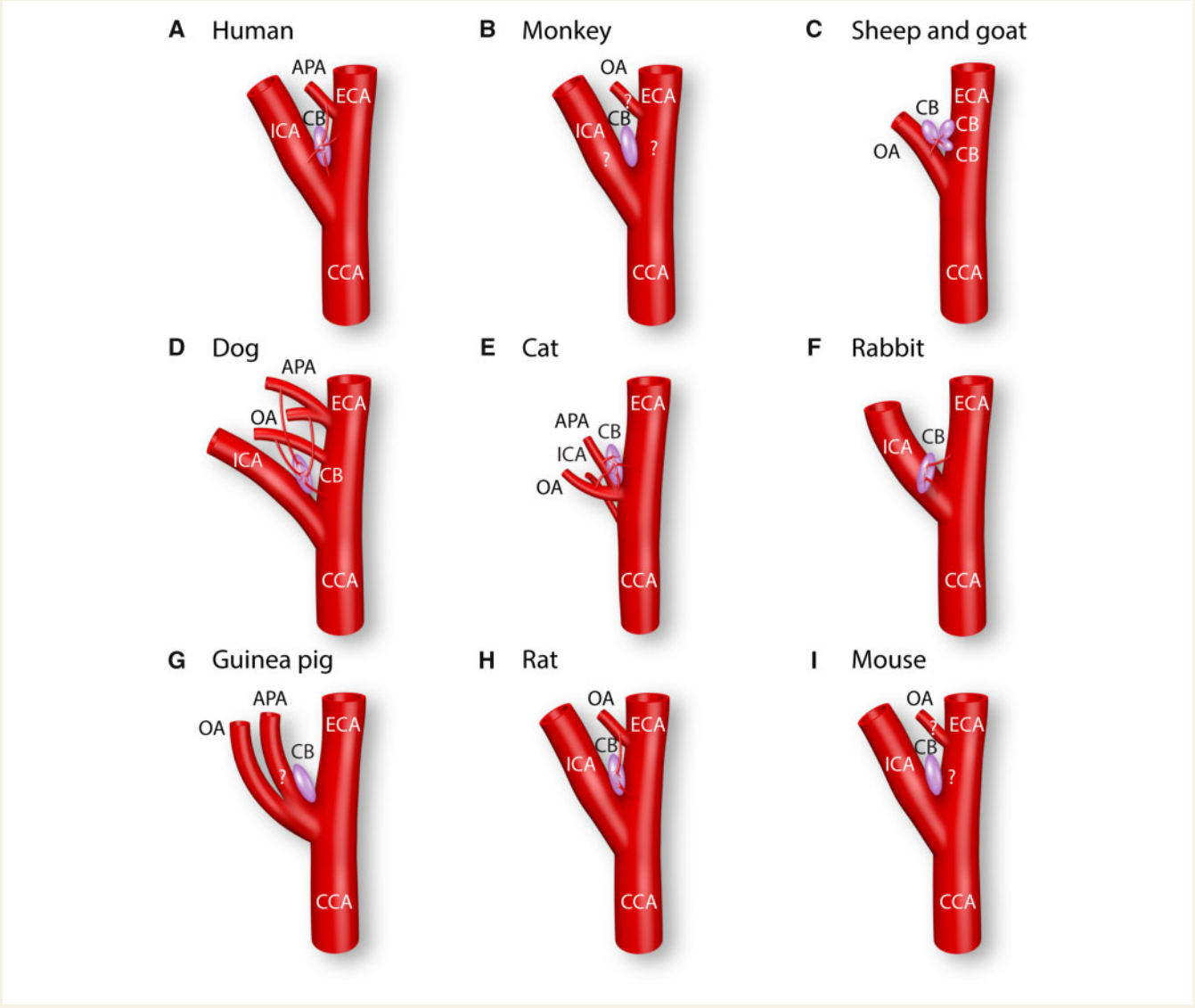

Figure 1 Species variation of the origin of CB blood supply. Schematic representation of the origin of CB blood supply in human (*A*), monkey (*B*), sheep and goat (*C*), dog (*D*), cat (*E*), rabbit (*F*), guinea pig (*G*), rat (*H*), and mouse (*I*). APA, ascending pharyngeal artery; CB, carotid body; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; OA, occipital artery. Data from Refs.[39–42,48–52,58–60,67]

distinct capillaries classified as Types I and II (*Figure 2*).[58,70] Type I capillaries are large (14–20 μm of diameter) and supply clusters of glomus cells forming intimate curved contacts around single glomus cells. They are connected to many collecting venules by channels, which are narrower than the capillaries themselves, and have numerous fenestrations, few caveolae and are only partially covered by pericytes. In contrast, Type II capillaries are smaller (~7 μm of diameter), do not supply glomus cell clusters, have both straight and curved segments and are covered by pericytes at their arterial end and endothelial fenestrations at their venous end.[58,70] They provide an alternate route for blood flow through the CB.[58] Both types of capillaries are preceded by precapillary sphincters, which in are composed of a complete layer of smooth muscle cells or pericytes, that contribute to controlling the blood flow from arterioles into capillary branches within the CB. Numerous arteriovenous anastomoses in the interior of the CB were found where arterioles connect directly to venules,[58] but their function remains unknown.

The capillaries are connected to venules at both the outer surface of the CB and within its interior. Some venules from the CB connect to either the internal or external jugular vein forming a CB venous plexus that contributes to its drainage. In addition, caudal and rostral projecting veins from the CB connect to the pharyngeal vein that drains into the external jugular vein via the pharyngeal plexus and the posterior facial vein. Finally, ventral veins join the internal jugular vein.[58] Therefore, arterioles and venules are the most important compartments for regulating total blood flow. This occurs via the presence of the aforementioned precapillary sphincters in terminal arterioles and narrowing channels at connecting points of arterial vessels with venules. Altogether, total blood flow through the CB is regulated by sphincters at the arterial and venous vessels.

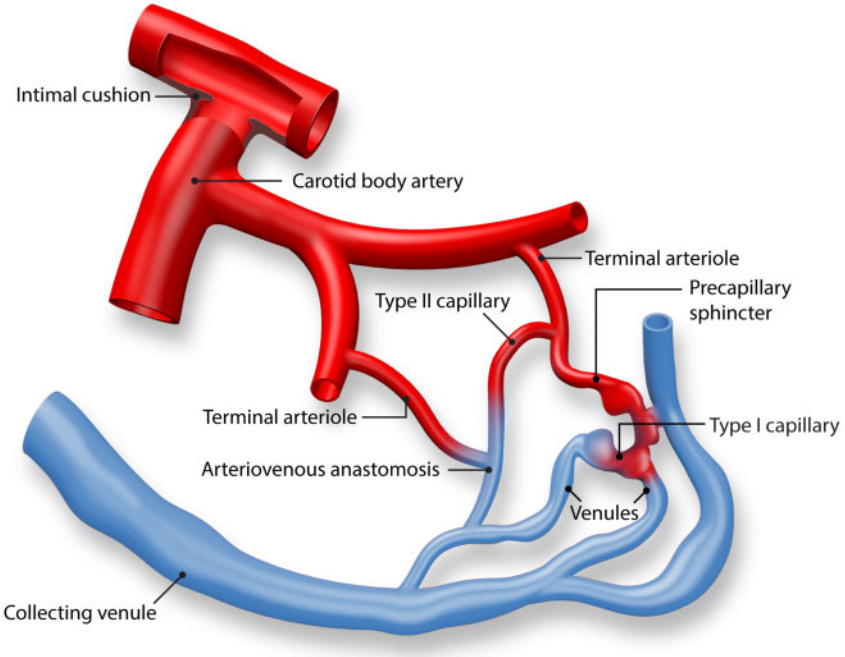

Figure 2 Schematic representation of the blood vessels within the rat carotid body. The carotid body artery enters the carotid body and its branches forming third- and fourth-order arterioles before arterioles form leading to capillaries, which are composed of two types: Type I penetrate a cluster of glomus cells and Type II that do not penetrate glomus cell clusters. The capillaries are connected to venules that drain into the internal and external jugular vein to compose the carotid body venous plexus. Figure adapted from Ref.,[58] permissions received.

4. The remarkable blood flow of the CB

In addition to the precapillary sphincters, another structure contributes to the control of blood flow within the CB known as the 'intimal cushion' (*Figure 2*). The intimal cushion is formed by circumferential smooth muscle cells, collagenous fibres, basal and elastic laminae, and also by components of the extracellular matrix.[58] It is found at the origin of the CB artery and can reduce the diameter of this artery by >50%, and this is most likely mediated through its autonomic innervation.[58,71] Nevertheless, despite this structure being found in rats, studies in humans do not report its presence.[72]

Relative to human brain (~50 mL/min/100 g)[73,74] and heart (~80 mL/min/100 g),[75–77] the CB has a relatively large blood flow compared to other highly metabolically active organs in the body, and the same is observed in other species, for instance, in cats.[37] In cats, the total CB blood flow (venous outflow) was reported to be 1417–2000 mL/min/100 g,[37,78] while in rabbits it is 700–1203 mL/min/100 g.[20,79] Clarke *et al.*[47] reported that in rats and non-human primates, the CB tissue-specific blood flow (blood flow measurement based on the wet weight of the dissected organ) is 104 mL/min/100 g and 31 mL/min/100 g, respectively.

In disease states, there is evidence that blood flow to the CB is diminished. With impaired cardiac output in heart failure, blood flow to the CB is reduced, and this diminution is associated with an increase in peripheral chemoreflex sensitivity and a hyperreflexic sympathetic response.[7,20,21] Ding *et al.*[20] showed that, in addition to the reduction in the carotid blood flow, chronic heart failure and carotid artery occlusion similarly decreased neural nitric oxide synthase (NOS) expression and nitric oxide (NO) levels in the CB, which might explain the raised reflex sensitivity, given the inhibitory role of NO in the CB.[9,10] In addition, angiotensin II type 1 receptor protein expression and angiotensin II concentration were both elevated in the CB of heart failure rabbits,[20] which may induce vasoconstriction. Furthermore, it has been suggested that the decrease in CB blood flow is associated with a reduction in expression of Kruppel-like Factor 2, which is induced by endothelial cell shear stress and associated with reduced NO bioavailability, elevated inflammation, and angiotensin metabolism.[7,21,22,80]Kruppel-like Factor 2 expression was decreased in chronic heart failure and sensitized CB function by: (i) decreasing endothelial NOS expression thereby reducing NO bioavailability; (ii) increasing angiotensin-converting enzyme 1 expression, which increases angiotensin II levels, consequently enhancing the renin–angiotensin system activity within the CB; and (iii) triggers inflammation since the Kruppel-like Factor 2 has anti-inflammatory function and inflammation has been associated with CB sensitization.[7,21,81–83] Whether there is also expression of adherence molecules by the CB endothelium trapping leukocytes, as was seen in the brainstem microvasculature of spontaneously hypertensive rats,[84,85] remains a possibility to be confirmed.

It is known that the bifurcation of the common carotid arteries is prone to developing atherosclerosis due to several factors which include the turbulence, the flow velocity changes and the artery wall stress among others.[86–88] The pioneering study of Lowe *et al.*[89] suggested that the progressive carotid atherosclerosis compresses the CB blood supply through the glomic arteries.[89] These authors[89] showed a progressive loss of glomic tissue in the CB as the carotid bifurcation arteries

(external, internal, and common) became increasingly stenosed with age. More recent studies also demonstrated damage to the CB damage with carotid artery stenosis.[90,91] Matturri *et al.*,[90] studied elderly patients who died from cerebral vascular disorders and had carotid artery obstruction; these patients presented atrophy and fibrosis of the CB associated with a focal decrease of CB vascularization.[90] Thus, severe damage of the CB and a reduction of the CB blood supply are characters of carotid artery stenosis. It is known that the ischemia of the CB increases peripheral chemoreflex sensitivity,[20] which causes significant mortality and morbidity in several diseases.[92,93] In addition, patients with asymptomatic carotid artery stenosis have increased chemoreflex sensitivity.[94] Further, carotid artery revascularization following endarterectomy reduced chemoreflex sensitivity in patients with arteriosclerotic carotid stenosis.[95] Compared with stenting, endarterectomy was more effective in reversing chemoreceptor function in these patients.[95]

Of note, given that in humans common carotid artery blood flow is reduced on standing compared with the supine position[96–98] this may provide a stimulus to the CB to increase its activity. Thus, changes in blood flow to the CB clearly affect its sensitization. This prompts the question regarding autonomic innervation of CB vasculature and whether changes in autonomic vasomotor nerve discharge can alter CB reflex sensitivity and tonicity.

5. Autonomic innervation of the CB and control of blood flow

Given the increased sympathetic vasomotor tone in hypertension, the question arises whether this is also increased in the sympathetic nerves innervating the CB arterioles and, if so, does this decrease its blood flow to a level sufficient to activate glomus cells. If so, this could trigger a systemic increase in sympathetically mediated vasoconstriction and hypertension, and possibly further vasoconstriction in the CB via a positive amplification feedback loop. To understand better this possibility, it is crucial to comprehend the autonomic innervation of CB vasculature and whether this is altered in disease states.

Autonomic innervation of the CB includes innervation of three structures: (i) all segments of the vascular bed—arterioles, capillaries, precapillary sphincters, intimal cushion, arteriovenous anastomoses, venules, and small venules—(*Figure 2*); (ii) type A glomus cells; and (iii) sympathetic and parasympathetic ganglion neurones located circumferentially within the CB.[71,99] It is well described that within the CB, one or two glomus cells are located alongside glial-like cells commonly adjacent to a capillary.[100] Furthermore, electron microscopy studies have identified two subpopulations of glomus cells (Type A and Type B) based on the diameter of their dense-core vesicles: Type A glomus cells contain larger dense-core vesicles than Type B. Type A glomus cells are the only cells that receive either afferent or efferent nervous innervation and are more abundant than Type B cells (*Figure 3*).[101]

Autonomic innervation of the capillaries is not completely understood. However, McDonald[71] discusses that they could be involved with vasomotor changes via direct effects on the extensive pericytes that exist on CB capillaries, or indirectly via actions on glomus cells. Nevertheless, according to the author,[71] they could also take up amines inflowing into the CB via the circulation.

5.1 Sympathetic innervation

The CB receives sympathetic postganglionic fibres from the SCG via the ganglioglomerular nerve (GGN).[100] The postganglionic sympathetic neurons exclusively innervate blood vessels.[71] Some of these sympathetic postganglionic cells are located outside the SCG either along the GGN or within the CB itself.[100] They receive inputs from preganglionic neurons ascending via the cervical sympathetic trunk (CST).[99] Similar to chromaffin cells within the adrenal medulla, the CB also receives preganglionic sympathetic innervation from the CST that directly innervates some glomus cells (*Figure 4A*).[99]

In cats, Eyzaguirre and Uchizono[108] reported that some sympathetic fibres originating from the SCG course within the CSN to innervate the CB. The authors described a thick nerve dividing into two branches, in which one of them pierced the glomerular capsule that surrounds the CB, whereas the other branch bypassed the CB and joined the CSN caudally. In rats, a similar description was conveyed by McDonald.[102] He described that a small branch of the CSN joined the sympathetic nerve that emerges from the SCG and alongside the GGN comprises the external carotid nerve. According to McDonald,[102] this nerve *emerged from the lateral aspect of the superior cervical ganglion, wrapped around the carotid sinus in a dorsal to ventral trajectory, passed just dorsal to the sinus nerve* [where they connected], *and then joined the sympathetic nerves on the external carotid artery* (Supplementary material online, *Figure S1A*). The author emphasized that at the convergence between the CSN and the sympathetic nerve was used as a thoroughfare for baroreceptor terminals to innervate the carotid sinus region. However, this anatomical description does not rule out the idea that postganglionic sympathetic neurons course to the rostral pole of the CB via the CSN as described by Eyzaguirre and Uchizono.[108] Whether the function of these nerves relative to the sympathetic fibres originating from the SCG differs, remains to be elucidated.

In cats, Biscoe and Sampson[103] reported two extra-cranial postganglionic branches from the SCG connecting the internal carotid nerve with the glossopharyngeal nerve (GPN). From these branches, postganglionic sympathetic neurons make a loop and return to the CB via the CSN (Supplementary material online, *Figure S1*). The authors recorded from the central end of fibres peeled from the CSN cut close to the CB that demonstrated rhythmic activity modulated by respiration. The spontaneous rhythmic activity of these postganglionic sympathetic fibres was abolished when the extra-cranial postganglionic branches from the SCG were cut. Therefore, in cats, postganglionic sympathetic fibres from the SCG join the CSN at two locations: caudal portion of the CSN and the GPN (*Figure 4A*and Supplementary material online, *Figure S1B*).

5.2 Parasympathetic innervation

This occurs via the CSN and a branch of the vagus nerve. The preganglionic neurons synapse with autonomic ganglion cells either just outside or inside the CB. Some authors refer to the set of these ganglionic cells as the carotid ganglion[109] and argue that they innervate blood vessels exclusively (for review see Daly,[100] p37 and McDonald and Mitchell[99]), whereas other authors do not accept this assumption and suggest they could also innervate glomus cells.[110] Biscoe *et al.*[111] reported that nerve endings found to synapse with some glomus cells are efferent neurons with soma located in the brainstem; this suggests a direct preganglionic parasympathetic innervation. Retrograde labelling revealed preganglionic parasympathetic cell bodies at two nuclei: the nucleus parvocellularis reticularis and the retro nucleus ambiguus (*Figure 4B*).[104] However, lately, the importance of preganglionic parasympathetic innervation has

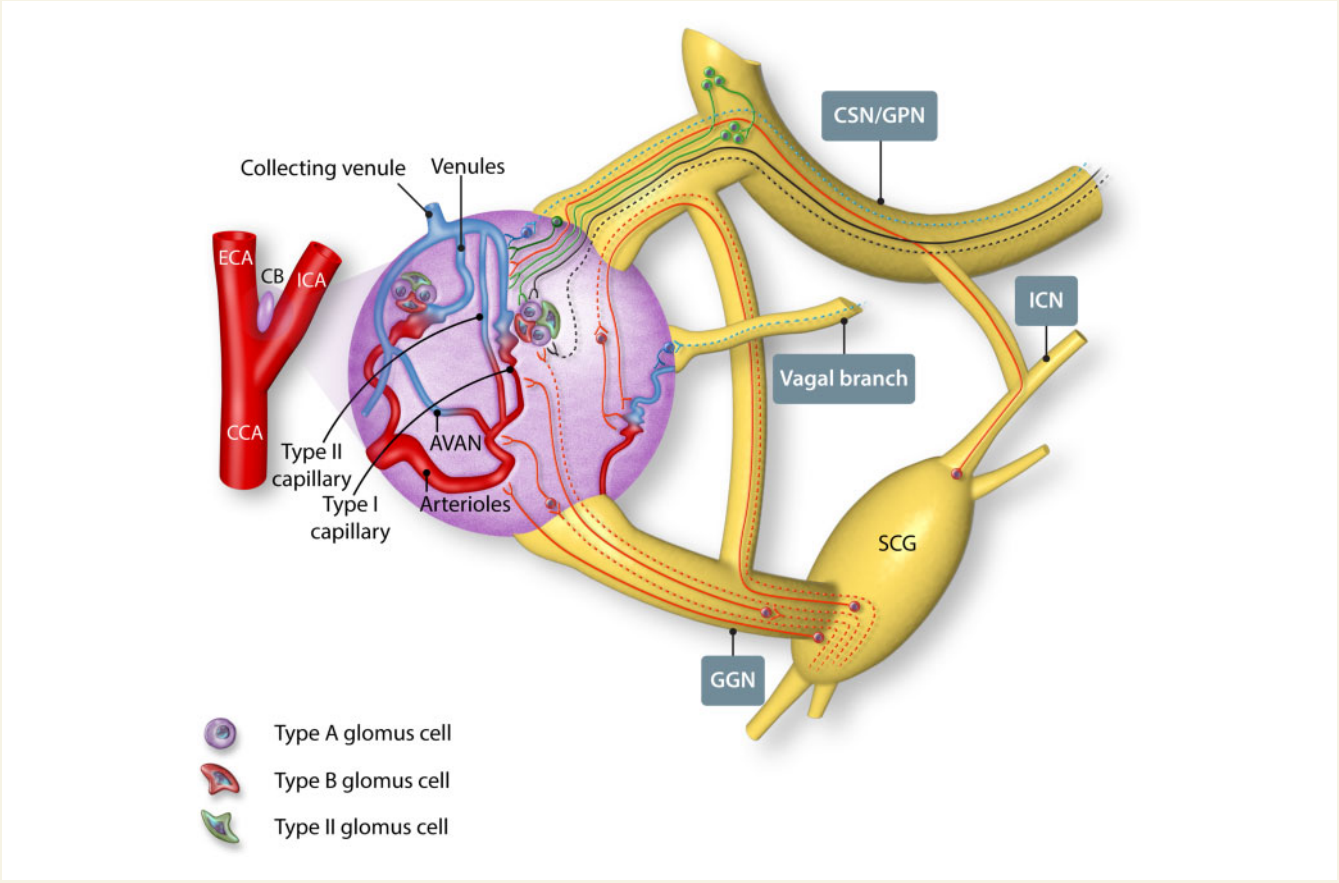

Figure 3 Superimposition of the autonomic innervation and circulations of the carotid body. The CB receives afferent (black), efferent—sympathetic (red) and parasympathetic (blue)—innervation. Within the CB, connections are made with two main structures: Type A glomus cells and vasculature (arterioles, capillaries, and venules). Type A glomus cells receive inputs from afferent neurons [tyrosine hydroxylase-positive (dashed line) and nitric oxide synthase-positive (continuous line)], NO-producing (green) paraganglia cells, and preganglionic sympathetic neurons (dashed line). In contrast, the vasculature is innervated by postganglionic sympathetic neurons (continuous line) traversing via nerve branches from the SCG and sympathetic ganglionic cells located at the peripheral surface and within the CB. Furthermore, parasympathetic innervation to the vasculature also originates from ganglionic cells and NO-producing ganglionic cells (dark green), which are positive to ChAT. The sympathetic fibres reach the CB via two nerves: the GGN and the CSN. The CSN receives sympathetic fibres via: (i) a branch originating from the GGN that bypass the CB joining the CSN at its caudal portion, and (ii) an extracranial branch that connects the ICN to the GPN (to date, shown only in cats). Preganglionic parasympathetic neurons (dashed line) reach the CB via the CSN and a branch from the cervical vagus nerve. AVAN, arteriovenous anastomosis; CB, carotid body; CBA, carotid body artery; CCA, common carotid artery; ChAT, choline acetyltransferase; CSN, carotid sinus nerve; ECA, external carotid artery; ECN, external carotid nerve; GGN, ganglioglomerular nerve; GPN, glossopharyngeal nerve; ICA, internal carotid artery; ICN, internal carotid nerve; NO, nitric oxide; SCG, superior cervical ganglion. Data from Refs.[58,71,99,102–107]

been questioned, especially with the report of putative innervation of paraganglia neurons present within both the GPN and CSN.[115] A summary of the parasympathetic innervation of the CB is depicted in *Figure 3*.

6. Autonomic influences on CB chemoreceptors: vascular, non-vascular, or both?

The function of sympathetic and parasympathetic innervation on CB afferent discharge has been studied in non-rodent species[100,106,114,115] (for review see de Burgh Daly,[100] p85–88) and is described next for each limb of the autonomic nervous system.

6.1 Sympathetic responses

McDonald and Mitchell[99] reported that preganglionic sympathetic connections of glomus cells represented only 1% of all connections; therefore, we can speculate that not all glomus cells are innervated and the bulk of effect of sympathetic activation to the CB is most likely mediated via a vascular effect. Floyd and Neil[116] were the first authors to evaluate the effects of sympathetic innervation on peripheral arterial chemoreceptor afferent activity. They electrically stimulated the CST in anaesthetized cats and reported an increase in chemoreceptor afferent discharge. Thereafter, Daly *et al.*[78] reproduced this finding and also showed an associated reduced CB blood flow and vasoconstriction with cervical sympathetic stimulation. Two decades later, McDonald and Mitchell[99] demonstrated preganglionic sympathetic innervation to some glomus cells and proposed that sympathetic innervation might have two distinct effects: (i) an excitatory effect on CB afferent discharge caused by vasoconstriction and reduced blood flow; and (ii) an inhibitory response

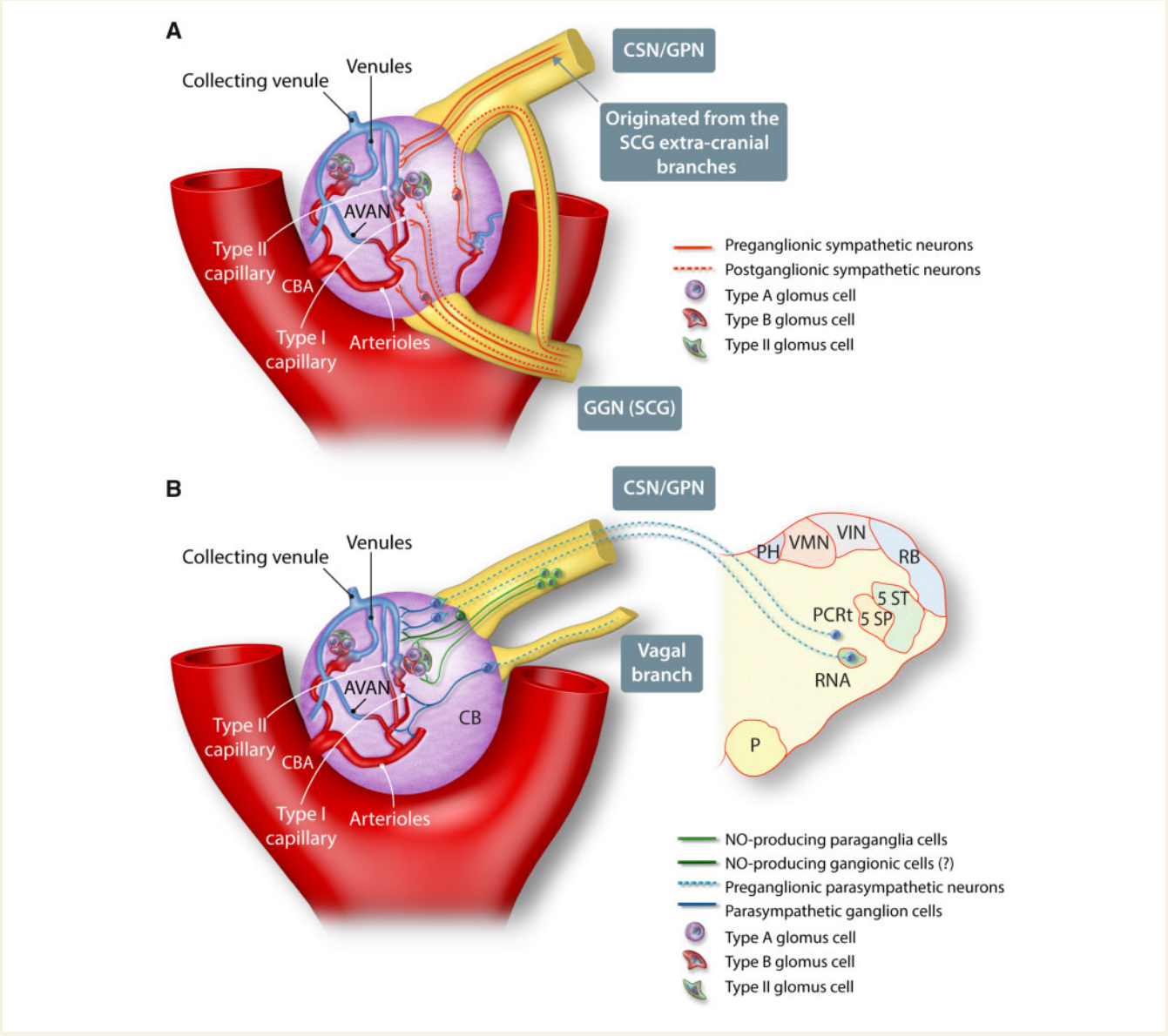

Figure 4 Intra-CB connections of sympathetic (*A*) and parasympathetic (*B*) nerves. (*A*) Within the CB preganglionic sympathetic neurons traversing via nerve branches from the SCG synapse with both Type A glomus cells and ganglionic cells located within and at the peripheral surface of the CB; the latter innervates the vasculature. In contrast, postganglionic sympathetic neurons located within the SCG project via the CSN and the GGN to the CB innervate the vasculature only (arterioles, capillaries, and venules). Data from Refs.[71,99,103] (*B*) Preganglionic parasympathetic neurons innervating CB are located within the brainstem at the PCRt and the RNA. These neurons project via the GPN and CSN as well as a branch from the cervical vagus nerve to the CB. They synapse with parasympathetic ganglion cells (blue) at the surface of the CB that are located adjacent to the entry/exit points of the CSN and cervical vagus nerves innervating the CB. These parasympathetic ganglion cells innervate CB arterioles and capillaries that are either adjacent to, or distant from, glomus cell clusters. NO-producing paraganglia (green) cells that are located within the CB (dark green) and along the CSN/GPN innervate the Type-A glomus cells and blood vessels; they are known to have an inhibitory function on CB afferent activity. 5 SP, nuclei of the spinal tract of V; 5 ST, spinal tract of V; AVAN, arteriovenous anastomosis; CB, carotid body; CBA, carotid body artery; CNS, carotid sinus nerve; GGN, ganglioglomerular nerve; GPN, glossopharyngeal nerve; P, pyramidal tract; PCRt, parvocellularis reticulares nucleus; PH, nucleus praepositus hypoglossi; RB, restiform body; RNA, retro nucleus ambiguus; SCG, superior cervical ganglion; VIN, inferior vestibular nucleus; VMN, medial vestibular nucleus. Data from Refs.[71,102,104,105,112]

mediated by an evoked release of dopamine via the innervation of glomus cells. Dopamine acting on D_2 receptors is well known to exert an inhibitory effect upon both chemoreceptors discharge and the hypoxic ventilatory response.[117,118] Pharmacological studies have shown that injecting cholinergic nicotinic agonists increase the release of norepinephrine (NE) in the CB.[119] It was suggested that nicotinic receptors (nAChR) are located in specific NE-containing glomus cells.[119] However, according to the study of McDonald and Mitchell,[99] the only chemoreceptors which are immunoreactive for dopamine beta-hydroxylase (i.e. able to produce NE) are the Type B glomus cells, and these receive neither afferent nor efferent innervation. One possibility is that Type A glomus cells are excited via preganglionic sympathetic fibres and

simultaneously depolarize Type B cells via gap junction connections.[99] Another source of NE via nAChR activation is released from sympathetic postganglionic cells located within the CB (*Figure 5*).[99]

Regarding the excitatory effect of the sympathetic innervation of the CB, O'Regan[120] suggested two mechanisms: the first mediated by the release of NE acting on vascular α_1-adrenergic receptors and the second produced by co-release of neuropeptides. In fact, postganglionic sympathetic neurons in the CB are known to co-release neuropeptide Y (NPY) with NE, and this one is able to produce vasoconstriction in other vascular beds.[119,121,122] In cats, intra-carotid injection of NPY was able to produce activation of hyperpnoea proving its ability to stimulate chemoreceptors[123]; however, whether or not glomus cells express NPY receptors is yet to be determined. Additionally, non-adrenergic neurons of sympathetic origin release vasoactive intestinal peptide (VIP).[109] VIP was demonstrated to activate afferent sensory fibres in the CB of cats and are known to produce vasodilation.[119,124] In the CB, NE causes vasoconstriction via α_1-adrenoreceptors located on vascular smooth muscle.[125] Acting via α_2-adrenoreceptors located on glomus cells, NE inhibits chemoreceptors decreasing their sensitivity.[119,126,127] The α_2-adrenoreceptors were also found on sympathetic endings where they modulate the

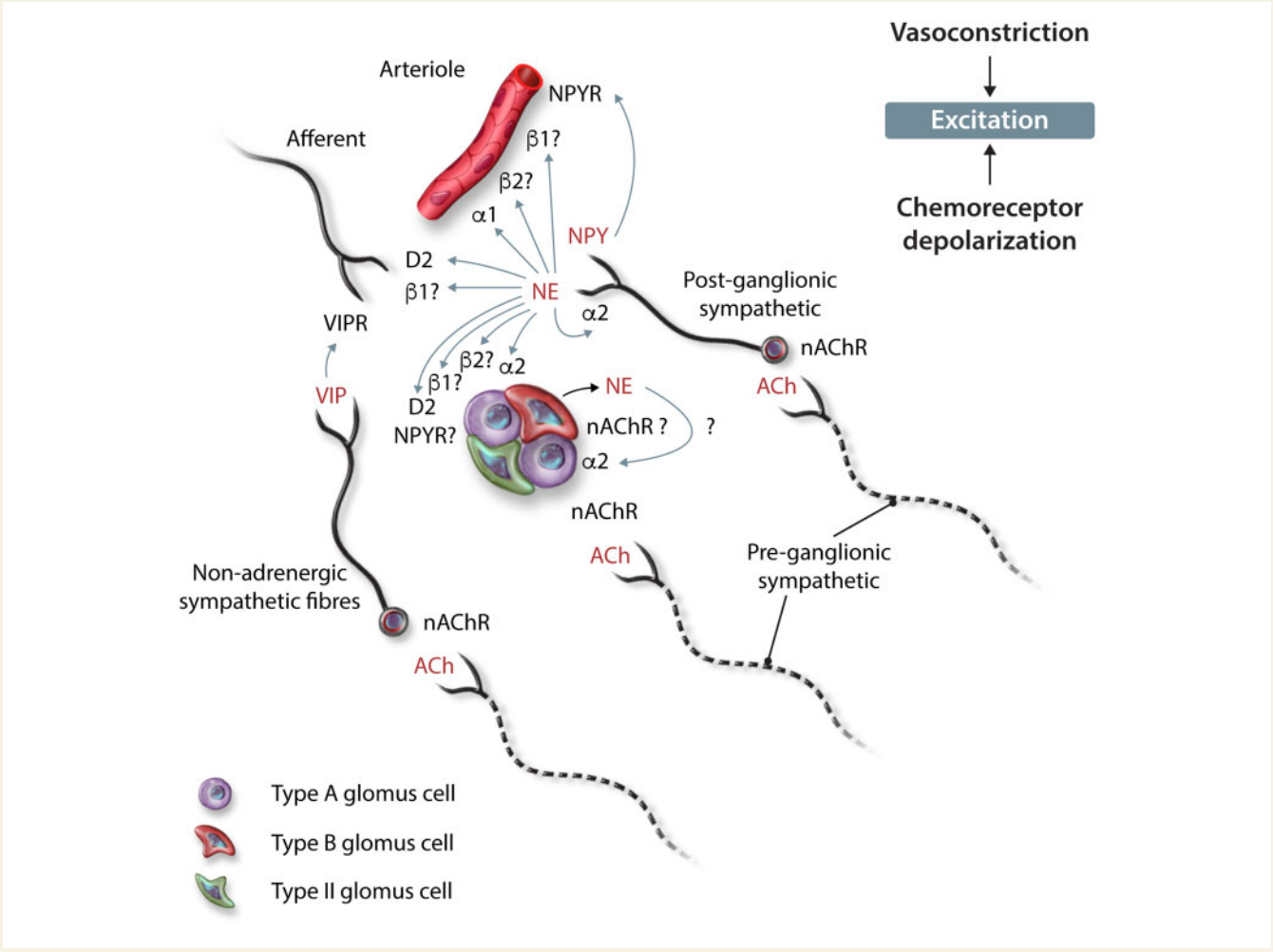

Figure 5 Proposed vascular and non-vascular effects for sympathetic efferents to the CB. Few glomus cells (~1%) receive direct preganglionic sympathetic innervation. CB blood vessels receive postganglionic sympathetic innervation from ganglionic cells whose soma are located either in the superior cervical ganglion, or throughout the ganglioglomerular nerve, or within the CB itself. It was suggested that nAChRs are located in specific NE-containing glomus cells (i.e. Type B glomus cells). However, these receive neither afferent nor efferent innervation. Another source of NE via nAChR activation is released from sympathetic postganglionic cells located within the CB. Postganglionic sympathetic cells in the CB co-release NPY, whereas non-adrenergic neurons of sympathetic origin release VIP. VIP was demonstrated to directly activate afferent sensory fibres in the CB and are known to produce vasodilation; conversely, NPY is known to produce vasoconstriction in other vascular beds; however, whether glomus cells express NPY receptors is yet to be determined. In the CB, NE causes vasoconstriction via α_1-adrenoreceptors located on vascular smooth muscle. Acting via α_2-adrenoreceptors located on glomus cells, NE inhibits chemoreceptors decreasing their sensitivity. The α_2-adrenoreceptors were also found on sympathetic endings where they modulate the release of NE. β_1-adrenoreceptors exert an excitatory effect on the CB, but whether these receptors are located in glomus cells, nerve endings of sensory fibres or vessels remains to be determined. Electrical stimulation of sympathetic innervation to the CB produces excitation, inhibition or no effect. These inconsistent responses might be explained by the ability of NE to bind to these different adrenoceptors. Note, NE is able to bind to inhibitory dopaminergic receptors (i.e. D_2 receptors) located on glomus cells and afferent sensory fibres. Furthermore, vasodilatatory β_2-adrenoreceptors might also play a role in the inhibitory effects of its sympathetic innervation. CB, carotid body; nAChR, cholinergic nicotinic receptor; NE, norepinephrine; NPY, neuropeptide Y; VIP, vasoactive intestinal peptide. Data from Refs.[99,109,119–122,124–130]

release of NE.[126] Folgering et al.[128] demonstrated excitatory effect on CSN discharge mediated via β_1-adrenoreceptors, but whether these receptors are located in glomus cells, nerve endings of sensory fibres or vessels are still to be determined. O'Regan[120] demonstrated that electrical stimulation of sympathetic innervation to the CB is able to produce excitation, inhibition, or no effect. These inconsistent responses might be explained by the ability of NE to bind to these different adrenoceptors. It is worth note that NE is able to bind, in some degree, D_2 receptors located in glomus cells and afferent sensory fibres.[129,130] Furthermore, vasodilatatory β_2-adrenoreceptors might also play a role in inhibitory effects of sympathetic (*Figure 5*).

6.2 Parasympathetic responses

The influence of the CSN innervation, which contains parasympathetic pre-ganglionic and paraganglia neurons, on CB chemoreceptor activity was investigated *in vivo*.[104,105,113,131] Measuring CB blood flow in cats, Neil and O'Regan[131] showed that stimulation of the CSN decreased chemoreceptor discharge and produced a concomitant increase in CB blood flow. Following intra-arterial injection of atropine close to the CB, the effect on the blood flow was abolished, but the inhibitory effect on chemoreceptor activity persisted. The authors came to the conclusion that the CSN-mediated inhibitory effect was due to a non-vascular effect. Later, McCloskey[132] criticized these findings due to lack of precautions to avoid pseudo-inhibition by antidromic depression of afferent discharge. Moreover, in contrast to Neil and O'Regan,[131] Goodman[133] found an inhibitory effect of CSN stimulation on chemoreceptors discharge that was abolished by close intra-arterial injection of atropine concluding that the CSN inhibitory effect was vasomotor in origin. Using different techniques, the same conclusion was reached by Belmonte and Eyzaguirre[134] and McCloskey.[132] The latter study tested two hypotheses: stimulating the efferent nerves to the CB will change chemoreceptors discharge via either (i) changes in blood flow or (ii) direct effects on glomus cells. The author used stagnant asphyxia to stimulate the chemoreceptors, which arrests blood flow. It was argued that if the effect of efferent innervation was mediated via glomus cells, then stimulating the CSN during stagnant asphyxia (when there is no blood flow) would continue to reduce chemoreceptor discharge. On the other hand, if the effect of CSN activity was mediated through changes in blood flow, then stimulating the CSN would generate no effect with the stagnant asphyxia protocol. The latter was the case. Therefore, the author claimed to prove that when there is no flow, there are no efferent effects; said differently, CSN-mediated modulation of CB afferent discharge is mediated via changes of its vascular resistance. An important caveat of this study, though, is the fact that McCloskey did not measure the CB blood flow itself. Furthermore, as thoroughly described by Jones[54] in his review of O'Regan's legacy, O'Regan[135] elegantly eradicated any concerns with antidromic pseudo-inhibition and proved the existence of non-vascular effects via activation of CSN efferent without electricity, demonstrating its inhibitory effect in the complete absence of blood flow.

Acker and O'Regan[136] electrically stimulated the CST and CSN and measured the CB total blood flow, local tissue blood flow, and PO_2 from within the CB in cats. The CB total blood flow was quantified via collection of the venous outflow from the transverse pharyngeal vein; the authors weighed the blood over periods of time to calculate the flow rates in μL/min. On the other hand, local tissue blood flow within the CB was measured in an indirect qualitative manner (hydrogen washout technique). The authors pierced the CB with two electrodes: with one electrode a small quantity of hydrogen gas was generated via electrolysis using constant current; the second electrode measured the hydrogen concentration in its immediate vicinity. Thus, *changes of blood flow in the vicinity of the tip of the hydrogen-sensing electrode caused a greater or lesser dissipation of hydrogen*. After confirming the chemoreceptor stimulatory (via sympathetic CST stimulation) and inhibitory (via parasympathetic CSN stimulation) effects on chemosensory afferent discharge, the authors administrated phentolamine (an α_1-adrenoreceptor antagonist) via close intra-arterial injection to block the effects of sympathetic stimulation. They observed that electrical stimulation of CST and CSN changed the chemoreceptors discharge with alterations of total blood flow; however, no variations on local tissue blood flow and glomeral PO_2 were seen. After phentolamine, changes in total blood flow following CST stimulation were abolished, but the effects on chemoreceptors discharge were attenuated only. The authors concluded that a possible non-vascular mechanism was involved. Of course, these findings do not rule out the possibility that other vasoactive neurotransmitters are being co-released with NE from sympathetic fibres, in which phentolamine would not antagonize. Modern imaging approaches could be useful to improve this information. Therefore, the evidence is inconsistent as to whether changes in blood flow consequent of autonomic nerve stimulation can affect CB afferent activity. Notably, there is a dearth of data regarding whether the sensitivity of chemoreflex evoked end-organ responses are affected by activation of autonomic nerves destined for the CB.

6.2.1 New players in the CSN efferent

McDonald and Mitchell[99,137] believe that electrical stimulation of the CSN leads to inhibition of CB chemoreceptors due to antidromic stimulation of afferent neurons forming 'reciprocal synapses' with glomus cells and release dopamine to depress their activity. This opposes the idea that stimulation of the CSN activates parasympathetic efferent fibres to enhance sensitivity. It is argued that ~95% of the afferent neurons that innervate the CB are located in the petrosal ganglion; 4% of petrosal ganglion neurons are immunoreactive for tyrosine hydroxylase; and 90% of these tyrosine hydroxylase positive neurons originate from the CB.[109,138] These neurons could be the source of the dopaminergic 'reciprocal synapses' proposed by McDonald and Mitchell.[99,137] We anticipate that CB afferents would operate in a similar way to that described for other sensory afferent fibres.[139] Peripheral afferents display significant arborization which could lead to depolarization at branch points where these peripheral afferent collaterals would send antidromic spikes back towards glomus cells. A second possibility is that the after-depolarization in petrosal ganglion neurones might generate an antidromic action potential. Both mechanisms would affect the sensitivity of the afferent ending as well as trigger transmitter release from the afferent terminal ending as has been described for muscle afferents which act to modulate their own excitability.[140] However, no functional data for such a mechanism exists for the CB, but that does not rule out that it may exist. On the other hand, an antidromic mechanism may not be the only way to achieve afferent sensitivity control. If dopamine is released from afferent terminals, this could be mediated by intracellular events triggered once the terminals are depolarized rather than orthodromic spikes generating antidromic ones. Also, if dopamine is released from afferent terminals, we do not know whether they will act on auto-receptors or will travel towards glomus cells (i.e. retrograde synaptic signalling).[141,142] If we consider that both blood flow and reciprocal afferent synaptic connections play a role in modulating CB sensitivity, it is possible that both authors are correct. For example, in Neil and O'Regan work,[131] atropine would block the effect on blood flow but not the release of dopamine from

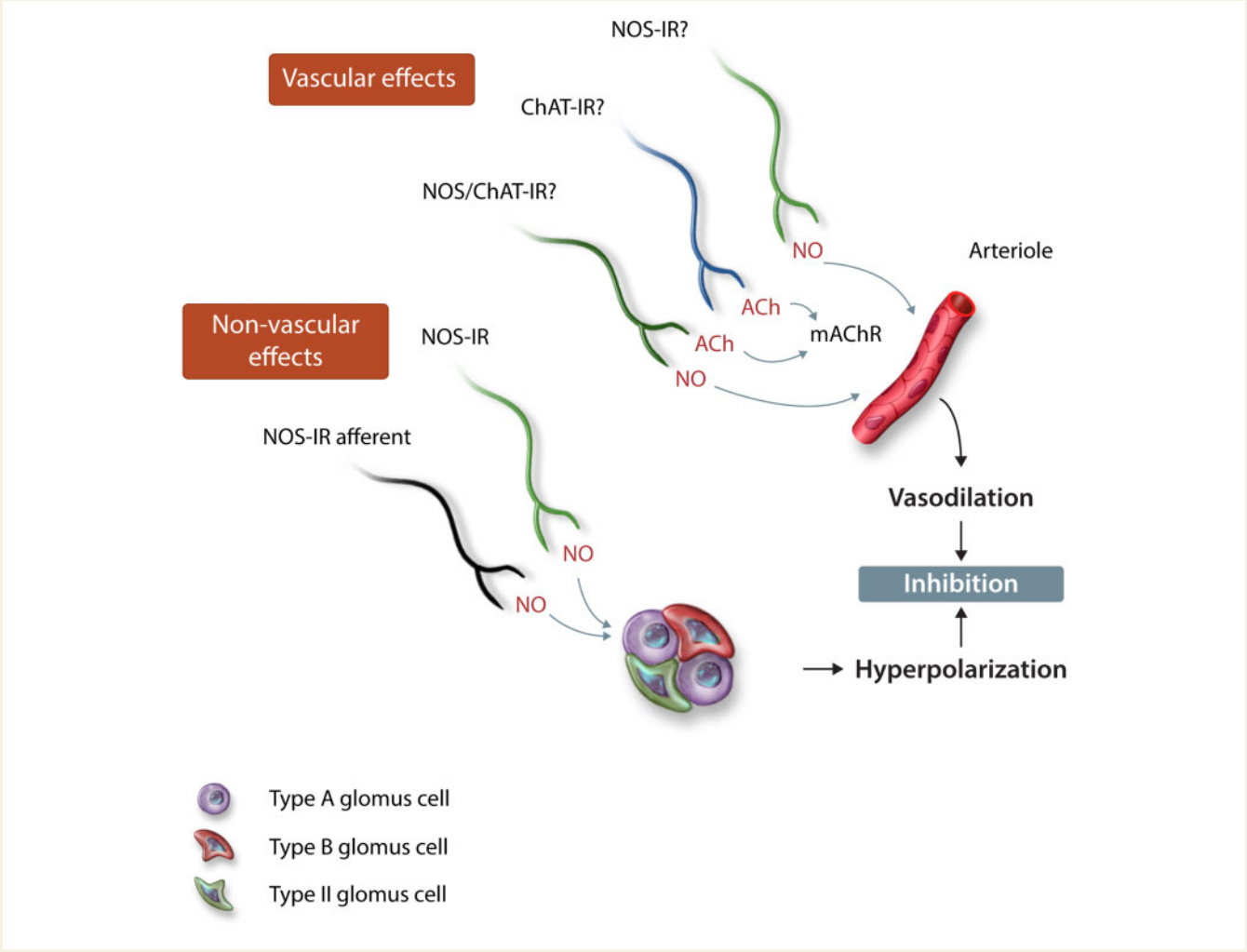

Figure 6 Proposed vascular and non-vascular effects for parasympathetic efferents to the CB. NOS-IR fibres from paraganglia cells located along the glossopharyngeal nerve and within the CB make contact with glomus cells and blood vessels in the CB. Some of these cells were reported to be positive for ChAT-IR; however, whether or not all cholinergic fibres are nitrergic is not clear. Putative NO released from NOS-IR fibres is involved with both vascular and non-vascular inhibitory effects of the parasympathetic system. Petrosal ganglion NOS-IR afferent fibres may be the main source of NO to glomus cells causing hyperpolarization. Conversely, the vascular effect of the parasympathetic innervation to the CB is not solely mediated by NO, but rather in association with ACh via mAChR. This was demonstrated via intra-arterial injection of atropine, close to the CB, to abolish the increase in CB total blood flow seen after electrical stimulation of the carotid sinus nerve. ACh, acetylcholine; CB, carotid body; NO, nitric oxide; NOS, nitric oxide synthase; mAChR, cholinergic muscarinic receptor. Data from Refs.[105,131,144]

reciprocal afferent connections. However, dopamine is not the only modulatory neurotransmitters proposed to be released from CSN efferent and sensory connections.

Wang *et al.*[143] demonstrated nitrergic fibres within the CSN and nitrergic varicosities juxtaposed to both blood vessels and glomus cells. Thus, stimulation of the CSN might also activate these nitrergic fibres and inhibit the CB; such an effect would not be blocked by atropine and may explain Neil and O'Regan[113,131] findings. Indeed, NO-mediated inhibition of CB could be explained by either an action on glomus cells or the vasculature causing vasodilatation and increasing blood flow (*Figure 6*). Wang *et al.*[143] addressed that most of the NOS-positive fibres that connect with glomus cells are sensory from the petrosal ganglion, whereas the ones innervating the vessels are autonomic. Additionally, they also reported that these cells are positive for choline acetyltransferase (ChAT); however, whether or not all cholinergic fibres are nitrergic is not known. All told, there is evidence suggesting that CSN innervation of the CB depresses chemoreception and improves blood flow, and can neuromodulate glomus cell activity (*Figure 6*).[136] However, to what extent and under what conditions NO and acetylcholine acts on the CB and whether the reductions in chemoreceptor afferent discharge are mediated by increases in blood flow to the CB is unclear.

An additional complexity is that paraganglia neurons residing in close proximity to blood vessels within the CSN/GPN are O_2 sensitive and activated by hypoxia.[145,146] With P2X2 and P2X3 purinoceptors present on these cells[107] one possibility is that adenosine triphosphate (ATP) released from erythrocytes within the CB during hypoxia could activate these NO producing paraganglia neurons providing a negative feedback loop to CB.[105,107] Whether this has a functional effect on afferent discharge or reflex response magnitudes remains to be investigated.

Table 2 Molecular pathways involved in the different functions of the carotid body

Molecular pathway	Physiological functionality	Where?	Physiological effect on excitability	What happens in disease state?	Associated disease (s)	Reference (s)
NOS/NO	Inhibits glomus cells	Varicosities fibres innervating glomus cells	↓	Down-regulation	Hypertension and heart failure	Ding *et al.*[20] Atanasova *et al.*[148]
Ang II and AT1R	Vasoconstriction and ↓carotid body blood flow	Vessels	↑	Up-regulation	Heart failure	Ding *et al.*[20]
KLF-2	↑NO and ↓inflammation	Endothelial cells	↓	Down-regulation	Hypertension	Fledderus *et al.*[83]; Iturriaga *et al.*[82]; Li *et al.*[81]; Marcus *et al.*[21]; Schultz *et al.*[7]
CO	Inhibits glomus cells	Red blood cells or glomus cells?	↓	Reduced	To be determined the importance in disease model	Prabhakar and Peers[10]; Prabhakar *et al.*[9]
H_2S	Inhibits Potassium channels and ↑$[Ca^{2+}]i$	Glomus cells	↑	Increased	To be determined the importance in disease model	Prabhakar and Peers[10]; Prabhakar *et al.*[149]
P_2X_3	Convey glomus cells sensory input to brainstem nuclei	Sensory fibres	↑	Up-regulation	Hypertension	Pijacka *et al.*[2]

↑ Increase.
↓ Decrease.

7. Clinical implications of dysfunctional CB autonomic innervation

As discussed above, emerging evidence supports a causal relationship between CB dysfunction and cardiovascular diseases such as heart failure and hypertension (*Table 2*).[18,150–155] Current data also suggest that this multi-modal receptor plays a role in the control of metabolism and glucose regulation, making it a potential target for diabetes.[156,157] In such diseases, the CB leads to augmented sympathetic outflow that will contribute to target organ damage (heart failure), total peripheral resistance (hypertension), enhanced chemical loop gain (apnoea), and glucose intolerance (diabetes). Studies have demonstrated that targeting the CB (denervation/resection) has beneficial effects in heart failure, hypertension and for improving glucose regulation in both humans and animal models.[2,17,18,27,34,156,160–163] CB hyperexcitability, which includes increased tonicity and hyperreflexia, is the apparent underlying mechanisms of dysfunction for the mentioned disease as demonstrated in spontaneously hypertensive rats, for instance.[2] Thus, hereafter, we will discuss how such hyperexcitability might be developed.

Accordingly, in some diseases, the CB leads to augmented sympathetic outflow that will contribute to target organ damage (heart failure), total peripheral resistance (hypertension), enhanced chemical loop gain (apnoea), and glucose intolerance (diabetes). The mechanisms behind CB dysfunction and its apparent hyperexcitability include increased tonicity and hyperreflexia as demonstrated in spontaneously hypertensive rats, for example.[2]

As recently proposed, hypoperfusion of peripheral organs will stimulate intrinsic afferent nerves (e.g. small diameter and unmyelinated) to cause reflex changes resulting in an imbalance in autonomic activity and elevated arterial pressure in an attempt to improve tissue blood flow.[164] As discussed above, in healthy animals autonomic innervation of the CB modulates chemoreceptors discharge: the sympathetic nervous system increased, whereas the parasympathetic nervous system decreased chemo-afferent discharge.[106,136] This might provide a rapid, dynamic, and reversible way to adjust chemo-afferent sensitivity under different physiological conditions. Although controversial, the modulatory effects of the autonomic system upon the CB may be directly related to changes in its blood flow.[20,165] Given this together with persistently elevated sympathetic activity in cardiovascular disease, the question is raised as to whether hyperexcitability of the CB results from an intense bombardment of sympathetic activity. This might also promote inflammation and vascular remodelling that further sensitizes the afferent output.

In pre-hypertensive spontaneously hypertensive rats, sympathetic overactivity is already present just after birth and takes the form of increased sympathetic-respiratory coupling.[166] Consequently, it is conceivable to assume that a reduction of CB blood flow via sympathetically mediated vasoconstriction within the body will lead to hypoperfusion and localized ischaemia, which could be responsible for the increased tonicity and sensitivity of these peripheral chemoreceptors (*Figure 7*). The underlying mechanism involved with this increased tonicity and sensitivity is not completely known. However, we can speculate on some possibilities based on previous comparable studies in Wistar and spontaneously hypertensive rats.

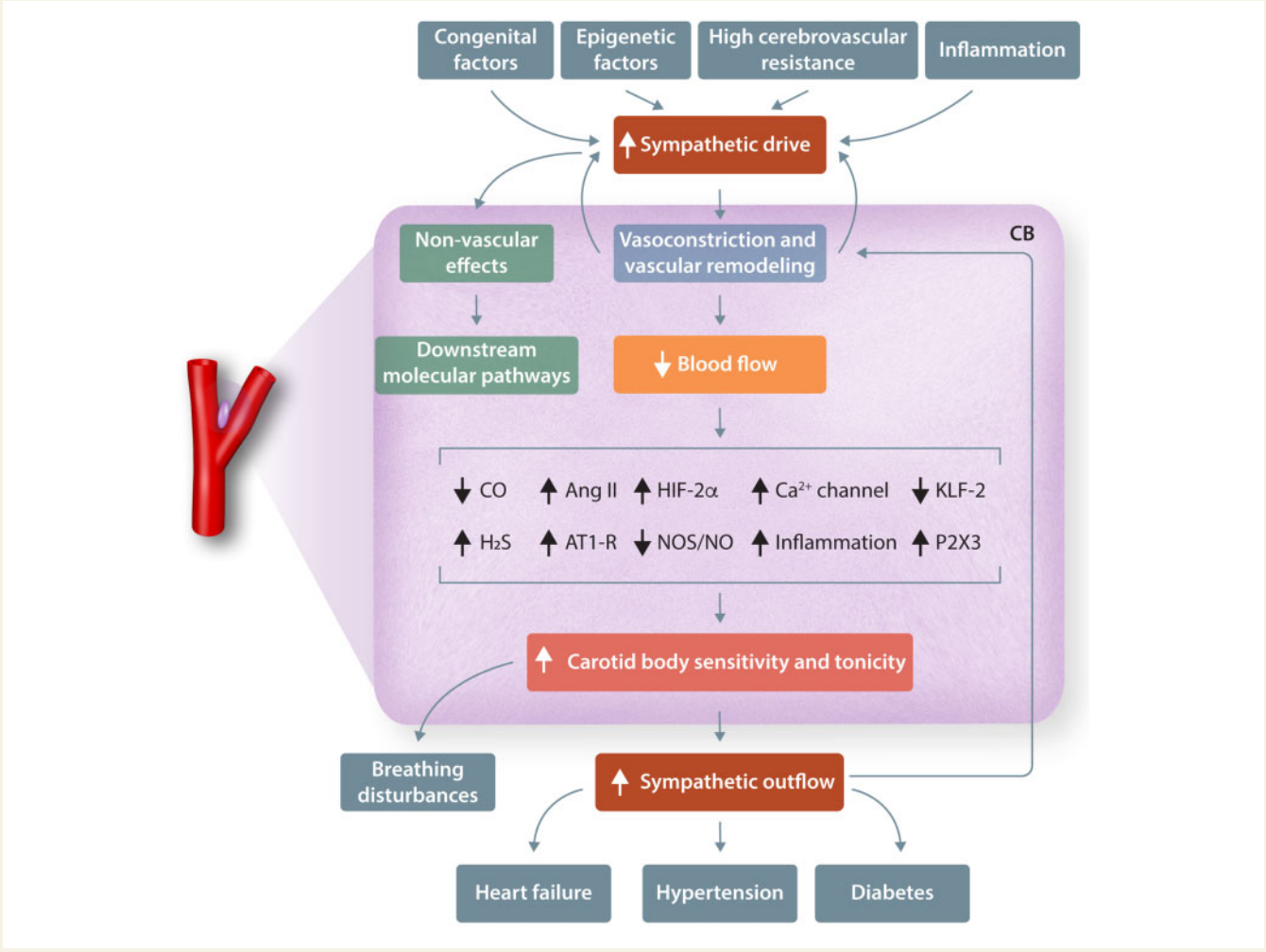

Figure 7 Schematic summary of some pathways associated with increased tonicity and sensitivity of the peripheral chemoreceptors. Congenital and/or neurogenic remodelling of the vertebrobasilar arteries might lead to brainstem hypoperfusion, increasing sympathetic activity. A reduction of carotid body (CB) blood flow via sympathetically mediated vasoconstriction within the body will lead to hypoperfusion and localized ischemia, which could be responsible for the increased tonicity and sensitivity of these peripheral chemoreceptors and contribute to the development and maintenance of disease as heart failure, hypertension, apnoea, and diabetes. The mechanisms by which hyperreflexia and aberrant tone of the CB are generated include: reduced carbon monoxide levels (CO), decreased neural nitric oxide synthase (NOS) expression and nitric oxide (NO), increased angiotensin II type 1 receptor (AT1-R) protein expression and angiotensin II (Ang II) concentration, enhanced HIF-2α, high levels of hydrogen sulfide (H_2S), up-regulation of the α_{1H} T-type Ca^{2+} channels, up-regulated expression of P2X3 purinoceptor, reduced Kruppel-like factor 2 (KLF-2) expression, up-regulated proinflammatory cytokines. The increased sympathetic drive also produces non-vascular effects which are involved with downstream molecular pathways. Data from Refs.[7,9,10,14,20–22,80–83,167,168]

7.1 Mechanisms of excitability in organs cells that share similarities with the SCG and CB glomus cells

Consideration is given to understanding mechanisms accounting for the elevated postganglionic sympathetic activity impinging on the CB and whether this might offer a plausible strategy to therapeutically (and indirectly) modulate CB sensitivity. In cultured rat adrenal chromaffin cells, signalling via brain-derived neurotrophic factor (BDNF) and its receptor tropomycin receptor kinase B (TrkB) were up-regulated during chronic hypoxia and this is dependent on hypoxia-inducible factor (HIF)-2α.[167] Pharmacological activation of TrkB receptors from hypoxic cells compared to non-hypoxic ones led to greater membrane excitability, increased Ca^{2+}-transients, and greater catecholamine secretion. Not only were more cells recruited for catecholamine release but also the quantal amount and frequency were increased.[167] This is most likely mediated by a greater entry of extracellular Ca^{2+} via the up-regulation of the α_{1H} T-type Ca^{2+} channels during hypoxia that are activated at potentials close to resting membrane potential.[169] Therefore, they will contribute to a low-threshold catecholamine discharge in response to mild depolarizations.[167] It is interesting that Arias *et al.*[170] demonstrated that neurotrophins, such as BDNF and nerve growth factor (NGF), are involved with the maintenance of ganglionic long-term potentiation (gLTP) in the SCG of rats. Such mechanisms may apply to the CB in cardiovascular disease and or to the SCG modulating effect; being therefore good targets to explore.

In the CB, the neurotrophins Ki-67, NGF, NT-3, and BDNF alongside their receptors (e.g. TrkB) were demonstrated to be up-regulated in

glomus cells of spontaneously hypertensive rats relative to Wistar rats.[171] Although speculative, we hypothesize that sympathetically mediated ischemia and chronic hypoxia within the CB would favour the formation of the HIF-2α and downstream signalling. One outcome would be up-regulation of neurotrophins and their receptors such as BDNF and TrkB together with T-type Ca^{2+} channels such as observed in chromaffin cells.[167] This would increase the membrane excitability of the glomus cells to low-intensity stimuli, therefore, increasing tonicity. In addition, the number of responding glomus cells recruited by stimuli, as well as the increased quantal amount and frequency of neurotransmitter (e.g. ATP) secreted during strong stimuli could also account for the hyperreflexia. Indeed, ATP, a major glomus cell transmitter, has been shown to play a major role in both CB tonicity and hyperreflexia.[2]

7.2 CB mechanisms of hyperexcitability

Long-term synaptic plasticity via neurotrophins is not the only possible molecular mechanism involved. In pre-hypertensive spontaneously hypertensive rats, overexpression of other receptors and channels have been demonstrated, for instance, the P2X3 purinoceptor and the ionic channels: amiloride-sensitive acid-sensing sodium channel (ASIC3) and 2-pore domain acid-sensing K^+ channel (TASK1) that might be or not involved with a hypoxia-induced molecular transduction in glomus cells.[2,8,172] CB sensitivity has also been shown to be elevated by inflammation and oxidative stress generation[82,173,174] as well as reduced NO-mediated efferent inhibition.[148]

As previously mentioned, the increased sympathetic-respiratory coupling is already present in young and pre-hypertensive spontaneously hypertensive rats (as is chemoreflex sensitivity and tonicity).[2] We propose that an initiating step is sympathetic over activity that sensitizes the CB, which via a positive feedback loop based on CB hyperexcitability amplifies sympathetic outflow further. Previous studies have demonstrated remodelling of vertebrobasilar arteries, that supply blood flow to cardiorespiratory brainstem nuclei of pre-hypertensive spontaneously hypertensive rats.[175] These changes lead to brainstem hypoperfusion, which in turn activated the Cushing response that may contribute to the development of hypertension via increased sympathetic activity.[176,177] Remodelling of vertebrobasilar arteries might be congenital and/or neurogenic in origin. However, a more recent study does not support sympathetic activity as responsible for this vertebrobasilar artery remodelling[178] but rather an immune response.[179] Whether or not there is a congenital remodelling of CB vasculature in hypertension or whether this is due to sympathetic hyperactivity or inflammation or their combination remains unknown.

In summary, unbalanced autonomic activity innervating the CB may be involved with its hyperexcitability due to a reduction in blood flow, leading to downstream genomic variation. Through sensitization of the CB, these changes will further enforce the development of hypertension, including elevations of sympathetic tone furthering the problem of CB hypoperfusion. There appear to be a host of possible new therapeutic targets that include reducing the sympathetic drive to the CB via autonomic denervation, pharmacological targets (e.g. P2X3, TrkB, ASIC3, TASK1), or sophisticated approaches, such as Optogenetics to control CB hyperreflexia in conditions of hypertension and heart failure. Selective targeting of the CB is one of the main issues. Endothelial glycocalyx is composed by proteoglycans and glycoproteins connected to different carbohydrate chains.[180] It is possible that the glycocalyx protein content of different vascular beds in different organs is unique, therefore, making it targetable via systemic delivery of drug/s. Whether the vasculature of the CB has a unique expression of recognition proteins within its glycocalyx is unknown but if it did it would allow targeted immunomodulatory therapy. Although this is horizontal speculation, it does deserve exploration especially given the unique nature of the circulation of this organ.

7.3 Chemoreceptors and COVID-19

Hyperexcitability of CB chemoreceptors is not the only clinical dysfunction we should consider. The inability of this organ to properly tackle hypoxaemia might generate severe consequences for patients. During the current pandemic outbreak of the novel 2019 coronavirus (COVID-19) caused by the virus SARS-CoV-2, some patients were reported to present what is called 'silent hypoxia'.[181] In this condition, oxygen saturation (SpO_2) as low as 60% were described but, remarkably, without loss of consciousness or any classical signs of hypoxia which are anxiety, confusion, and restlessness; instead, the patients remain calm.[182] We currently discussed[112] that coronavirus neurotoxicity—evidenced by the loss of taste- could also be interfering with the ability of CB chemoreceptors to sense hypoxaemia since both afferent modalities are mediated by the petrosal ganglia.

8. Conclusions

This review summarizes current information about CB vascularization in different species, its autonomic innervation, and clinical implications of its dysfunction. We provide insights into how autonomic mechanisms may control CB sensitivity and conclude that there is no consistent origin of the arterial feed to the CB, either between or within a species. It is clear that many questions remain to be answered about the function of both the sympathetic and parasympathetic innervation of the CB. For example: is the sympathetic innervation to the glomus cells preganglionic and/or postganglionic? Does the same fibre innervate arterioles and glomus cells or are there distinct fibres? Are the NO-producing paraganglia cells and the parasympathetic ganglion cells the same or distinct populations, and do they all receive inputs from the brainstem preganglionic neurons? Finally, can an autonomically mediated change in blood flow within the CB alter glomus cell-petrosal ganglion excitability? We believe resolving these questions will further our understanding of the physiological function of the CB and, consequently, help reveal pathological mechanisms that may be therapeutically targeted to treat numerous diseases.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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