Carotid body chemoreceptor function: Hypothesis based on a new circuit model

(chemoreceptive cell/stimulus–secretion coupling/dopaminergic interneuron)

EVA B. KRAMMER

Institute of Anatomy II, University of Vienna, Währingerstrasse 13, A-1090 Wien, Austria

Communicated by Paul A. Weiss, January 30, 1978

ABSTRACT Integration of our own morphological observations into recent ultrastructural, biochemical, and neuropharmacological results on the carotid body led to a new hypothesis on chemoreceptor function: (i) Glomus cells with small dense-cored vesicles (type IB cells) that store norepinephrine are chemoreceptors. (ii) Glomus cells with large dense-cored vesicles (type IA), which are postsynaptic to the other glomus cell type and presynaptic to afferent nerve endings, are dopaminergic interneurons that suppress the afferent discharge frequency during normoxia by releasing dopamine. (iii) The hypoxic stimulus causes the chemoreceptive cell to release the stored norepinephrine, which in turn brings about disinhibition of the afferent nerve endings by inhibition of the interneuron. (iv) Afferent nerve endings and interneurons interact through reciprocal synapses that form a short inhibitory feedback loop. We propose that information in the carotid body is processed in a fashion graded rather than digital, providing a fine adjusted cooperation of all elements.

Innervation of glomus cells: Afferent and/or efferent?

For about 50 years, since De Castro (1) ascribed a chemosensory function to the carotid body (an ascription confirmed by Heymans et al. (2)), there has been much controversy and puzzlement over the identification of the chemoreceptive element and the exact mechanism whereby a hypoxic stimulus is translated into stimulation of the carotid sinus nerve (CSN).

Each component of the carotid body parenchyma has been assumed to be the chemoreceptive one: glomus (type I) cells (1, 3–6), sustentacular (type II) cells (7, 8), “free” nerve endings (9), and nerve endings terminating on the type I cells (10, 11). Furthermore, in addition to having a chemoreceptive function, the glomus cells have been considered to be secretory or effector cells (9, 12–14), interneurons (10, 11), or a combination of these.

Perhaps the most generally accepted theory was that glomus cells respond to hypoxia by releasing a neurotransmitter that initiates an increase of firing rate of the nerve fibers terminating on the glomus cells (15). The afferent nature of these fibers was deduced by De Castro (16) from light-microscopic examinations. However, the early ultrastructural studies failed to show evidence of afferent synapses with the glomus cells. On the contrary, these nerve endings were thought to be presynaptic to glomus cells and therefore efferent in function, because they contain large numbers of clear vesicles (17–21). These electron microscopic results were further substantiated by Bisceo et al. (13, 22), who reported that nerve endings on glomus cells degenerate after the glossopharyngeal nerve is cut central to the petrosal ganglion. From these results, they concluded that the nerve endings belonged not to afferent axons whose cell bodies were in the petrosal ganglion, but to efferent axons whose cell bodies were in the brain stem. They concluded also that chemoreceptive nerves ended not on glomus cells but elsewhere in the carotid body. These findings and conclusions led to an alternative theory of chemoreceptor function (9): The chemosensory function was attributed to “free” nerve endings found in the carotid body. Glomus cells and associated nerves are held to form an efferent inhibitory feedback system, in which release of catecholamines, which are known to be present in large quantities in glomus cells, depresses chemoreceptor activity.

But meanwhile, other investigators (5, 11, 23, 24) have shown that decentralization of the IXth cranial nerve does not result in degeneration of any nerve endings on glomus cells. However, cutting the glossopharyngeal nerve peripheral to the petrosal ganglion results in more than 95% reduction in number of nerve endings on glomus cells (11). The remaining 5% of nerve endings belong to preganglionic efferent axons from the cervical sympathetic trunks; such axons enter the carotid body together with axons from the superior cervical ganglion (11).

In addition, recent ultrastructural studies of rat (11) and duck (4–6) carotid body have confirmed our observations in rat and mouse (E. B. Krammer and M. Lischka, unpublished observations) that most of the afferent nerve endings are postsynaptic to glomus cells (Fig. 1) and some of them form reciprocal synapses with these cells. Furthermore, adjacent glomus cells are interconnected by synapses (ref. 11; E. B. Krammer and M. Lischka, unpublished observations).

Therefore, the present ultrastructural evidence is strongly indicative of the glomus cells being part of an afferent system. However, the fact that glomus cells are presynaptic to afferent nerve endings is consistent with two functional alternatives: Glomus cells may function either as receptors of the hypoxic stimulus or as modulators of the chemoreceptive nerve endings.

Putative neurotransmitters in carotid body

Instead of yielding more evidence leading to understanding of carotid body function, neuropharmacological experiments contributed to further confusion.

Eyzaguirre and Zapata (25) have shown that intra-arterially applied acetylcholine (AcCho) increases the rate of chemosensory afferent discharge. They therefore suggested that glomus cells release AcCho in response to hypoxia, and the AcCho in turn stimulates afferent nerve endings. This hypothesis has been questioned as well (9, 26), because cholinergic (curariform) blocking agents indeed abolish the effects of applied AcCho, but do not affect the chemoreceptive response to natural stimuli (26–28). Moreover, though there is evidence for

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CSN, carotid sinus nerve; AcCho, acetylcholine; NE, norepinephrine.
the occurrence of AcCho (25, 29–31) and acetylcholinesterase (32, 33) in the carotid body, glomus cells do not store AcCho.

On the contrary, the occurrence of catecholamines in glomus cells has been shown by fluorescence histochemical methods (see refs. 9, 21, 34, 35). Biochemical evidence indicates that glomus cells contain large amounts of dopamine (1950 pmol/mg protein) and norepinephrine (NE) (1140 pmol/mg protein) (36). Most probably dopamine and NE are stored in different glomus cells (36–39), because—amongst other indications—dopamine-β-hydroxylase is contained in a special type of glomus cells, distinct from the most common dopaminergic glomus cell (40). These different types of catecholamine-storing cells might well correlate to the two kinds of glomus (type I) cells, which are distinguished morphologically, and in particular morphometrically by the diameter of their dense-cored vesicles (11, 41; M. Lischka and E. B. Krammer, unpublished data).

Type IA cells usually have a smooth, globular contour and contain a large number of large dense-cored vesicles (11), which might store dopamine (39, 41), the predominant amine of the carotid body of the rat. The type IB cells have an irregular contour with several long thin processes. They contain dense-cored vesicles nearly 30% smaller than those in type IA cells (11) and most probably store NE (39, 41).

Dopamine, when injected intra-arterially, inhibits sponta-

neous activity in CSN afferent fibers (42–44) and suppresses afferent discharge, even in the absence of blood flow (45, 46). This inhibitory effect of dopamine is reduced or abolished by pretreatment with dopaminergic or α-adrenergic blockers (45).

Current hypotheses on chemoreceptor function

Based on these recent ultrastructural, biochemical, and neuropharmacological data, two current theories for the receptor mechanism of the carotid body have been put forward.

Butler and Osborne (4–6) favor the idea that the glomus cells are the chemoreceptors. They propose that the afferent nerve endings are spontaneously active. During normoxia the discharge frequency is suppressed by continuous release of the inhibitory transmitter, dopamine, from the glomus cells at the afferent synaptic contacts. During hypoxia, the secretion of dopamine is reduced, allowing the nerve endings to depolarize. This depolarization, apart from increasing the discharge frequency, initiates release of a neurotransmitter, probably AcCho, from the efferent synapses of the afferent nerve endings. This efferent transmitter further suppresses dopamine release from glomus cells, which results in further depolarization of nerve terminals and an even greater increase in discharge frequency. Thus, the authors envisage a positive feedback loop operating at the reciprocal synapses of the afferent nerve endings.

McDonald and Mitchell (11) postulate that afferent nerve endings are chemoreceptors. Glomus cells and afferent nerve endings interact through reciprocal synapses that form an inhibitory feedback loop: sensory nerves release an excitatory transmitter during hypoxia, the transmitter causes glomus cells to release dopamine, and dopamine inhibits the sensory nerves. These authors consider the glomus cells not as chemoreceptors but as dopaminergic interneurons that modulate the sensitivity of chemoreceptive nerve endings. Preganglionic sympathetic nerve fibers also decrease chemosensory activity by enhancing dopamine release from some glomus cells. The postulate of Butler and Osborne, that the chemoreceptive glomus cell is continuously releasing during normoxia, is not in accordance with our assumption that nature functions economically. Shouldn't the function of the receptor cell be seen within the framework of the more general concept of stimulus–response (secretion) coupling? There are also several observations in disagreement with the hypothesis of McDonald and Mitchell that afferent nerve endings are chemoreceptors: After destruction of glomus cells by local freezing, and allowing sufficient time for complete nerve regeneration, chemoreceptor afferent activity was no longer observed (47). These results are complementary to those of Zapata et al. (48), who reported that following crushing of the CSN, recovery of chemosensory function depends on the reestablishment of apposition between regenerating nerve fibers and glomus cells.

But most surprising is the fact that Butler and Osborne did not know, and McDonald and Mitchell did not take into account, the existence of two distinct types of glomus cells. Thus, both working groups failed to integrate in their hypotheses the presumably noradrenergic type IB cell, a cell that differs in some remarkable respects from the type IA cell: most of these cells are presynaptic to type IA cells (11), they are negligibly innervated (11), and they have long processes that abut capillaries.

Our hypothesis: Function of glomus cells in chemoreception

We suppose that the discrepancies of interpreting the function of glomus cells result to some extent from considering the glo-
mus cell as either receptive element or interneuron, instead of assuming that each glomus cell type may have a different function.

Thus, we propose the following hypothetical circuit model (Fig. 2): Type IB cells, most of them presynaptic to type IA cells, are chemoreceptors that release NE in response to the adequate stimulus (i.e., hypoxia). Type IA cells, presynaptic to afferent nerve endings, are dopaminergic interneurons that suppress the afferent discharge frequency during normoxia by releasing dopamine. During hypoxia, NE brings about disinhibition of the afferent nerve endings by inhibition of the interneuron. Afferent nerve endings and interneurons interact through reciprocal synapses that form a short inhibitory feedback loop. Preganglionic sympathetic nerve fibers enhance dopamine release from some type IA cells, thus suppressing afferent discharge frequency. Synaptic interconnections enable glomus cells to communicate with one another.

**Type IB cell as chemoreceptive cell**

Besides the observation that most of the type IB cells are presynaptic to other glomus cells, the fact that the type IB cells are negligibly innervated attracted our attention.

From spectrophotometric and fluorometric studies there is evidence for the existence of two cytochrome $a_3$ components in the carotid body (7, 8). One has a low affinity for oxygen and is maximally reduced at an $O_2$ pressure of 7–9 mm Hg (930–1200 Pa). An increase in its reduction state precedes the rise in chemoreceptor discharge with falling $O_2$ pressure. The second component has a normal high oxygen affinity, is highly oxidized at an $O_2$ pressure of 7–9 mm Hg, and is maximally reduced in complete anoxia. The results of Mills indicate that the NAD component in glomus structures accessible to CSN stimulation is part of an electron-transport chain that has a normal cytochrome $a_3$ as terminal oxidase (7). Assuming that all glomus cells are innervated, Mills and Jóbis concluded that the low-affinity cytochrome $a_3$, not accessible to CSN stimulation, is localized in sustentacular (type II) cells. They also concluded that this low-affinity cytochrome makes chemoreceptors sensitive to hypoxia, and that therefore sustentacular cells function as an oxygen sensor.

According to our model, we propose that the low-affinity cytochrome $a_3$ is localized in type IB cells, because they are just as inaccessible to CSN stimulation as sustentacular cells. When the cytochrome is in its reduced state due to lack of oxygen, oxidative phosphorylation is depressed. Mills and Jóbis (8) suggest that this metabolic depression is followed by the release of a substance (potassium) that stimulates adjacent afferent axons. We find an alternate possibility more attractive: There is evidence that metabolic depression, i.e., the lack of high-energy phosphates, causes the release of $Ca^{2+}$ from mitochondria (49). This elevation of cytosolic $Ca^{2+}$ might initiate excocytosis (50, 51) of the stored NE from type IB cells during hypoxia.

Our hypothesis is in accordance with the observation that the content of NE + epinephrine in carotid body decreased during hypoxia and increased above the air control level when inspired $O_2$ concentration was raised to 40% (52). Unfortunately, the effects of applied NE are difficult to interpret. While dopamine inhibits carotid chemosensory discharge, this effect is not shown by NE, which produces an increase in afferent discharge in the in situ preparation, where effects upon the carotid body vessels cannot be eliminated (44, 46).

Although the interpretation of the fact that applied AcCho stimulates CSN afferent discharge is still obscure, we suppose that AcCho probably acts by depolarization of the noradren-

---

**Fig. 2.** Synaptic interconnections in the carotid body. Arrows indicate the direction of synaptic transmission and nerve conduction. Synapses interconnecting both types of glomus cells (type IA and type IB cell) are illustrated. Most of the type IB cells are presynaptic to type IA cells. A calyceiform afferent ending of the IXth cranial nerve (IXth) forms reciprocal synapses with a type IA cell. Some type IA cells are postsynaptic to efferent endings of preganglionic sympathetic nerves (PSN).

---

dergic type IB cell (analogous to the adrenal medullary cell), thus causing excocytosis by $Ca^{2+}$ entry instead of mobilization.

Curariform blocking agents might stabilize the type IB cell membrane and thereby counteract the action of AcCho on CN5 discharge. But these agents might fail to abolish the response to natural stimuli and to inhibitors of electron transport and oxidative phosphorylation, because thereby excocytosis is mediated by mobilization of $Ca^{2+}$ from intracellular pools.

**Type IA cell as interneuron**

There are several lines of evidence consistent with the concept that the type IA cells are the dopaminergic ones that are the final common path for the afferent nerve endings.

The stimulant effects of intraintravenous injections of AcCho and NaCN can be attenuated or abolished by simultaneously applied dopamine (46), while the inhibition by dopamine is reduced or abolished by pretreatment with dopaminergic or $\alpha$-adrenergic blockers (46, 53). Furthermore, recent experimental results (53) indicate that the inhibitory effects of dopamine on CN5 afferent discharge are due to hyperpolarization of afferent nerve endings.

From the input–output synaptic relationship we infer that not all glomus cells (11), but only type IA cells, are interneurons. Type IA cells receive synaptic input from three sources, because they are postsynaptic to type IB cells and to some efferent preganglionic sympathetic nerve fibers, and are postsynaptic to afferent nerve endings at their efferent synaptic contacts. And they are presynaptic at afferent synapses of afferent nerve endings. In this respect the type IA cell represents an integrative center rather than a simple relay station. This cell type might integrate excitatory and inhibitory messages before deciding whether or not to release the inhibitory transmitter dopamine to the postsynaptic membrane.

There exist several monoaminergic interneurons that re-
semble type IA cells morphologically: the small intensely fluorescent cells of sympathetic ganglia (see refs. 54–60), the amacrine cell of the retina (see refs. 61–64) and the granule cell of the olfactory bulb (see refs. 65, 66). Like type IA cells, in some species, these cells are dopaminergic and collect synaptic inputs from a variety of sources. The integrated result of these inputs is fed into the postsynaptic element as an inhibitory control over their activity.

The type IA cell has a peculiar resemblance to the granule cell, which is in fact a final common path for inhibition of the mitral cell. Disinhibition of the mitral cell can be brought about only by synaptic inputs inhibitory to the granule cell (65).

If there exist similarities beyond morphological ones between the granule cell and the type IA cell, the question arises at which synaptic contact the type IA cell could be inhibited to bring about a disinhibition of afferent nerve endings.

There are clear hints that type IA cells cannot be inhibited either at efferent synapses of preganglionic sympathetic fibers or at efferent synaptic contacts of afferent nerve endings. Previous studies have shown that hypoxia increases the rate of centrifugal discharge in the CSN (67). Electrical stimulation of the CSN suppresses chemosensory discharges (recorded from a small strand split from the same nerve) (68–71) by increasing both synthesis and release of dopamine from the glomus cells (43, 72, 73).

This depression of chemosensory activity, known as “efferent inhibition” of chemoreceptors, could be mediated not only by orthodromic stimulation of efferent sympathetic fibers, but also by antidromic invasion of afferent nerve endings connected to type IA cells by efferent synapses. Even if there exist two possible neuronal pathways that might give rise to “efferent inhibition,” the results demonstrate that stimulation of nerve fibers, connected to glomus cells by synapses, causes the type IA cell to release its inhibitory transmitter dopamine. Moreover, this electrically evoked inhibition of chemoreceptor discharge is blocked by drugs that abolish the inhibitory action of intra-arterially injected dopamine (43).

Thus, inhibition of type IA cells could take place only at the third kind of efferent synapses, where the type IA cell is postsynaptic to the type IB cell.

Joining together this deduction and the concept of stimulus–secretion coupling, we propose the following hypothesis: The hypoxic stimulus causes the type IB cell to release NE, which in turn brings about disinhibition of afferent nerve endings by inhibition of type IA cell.

Negative feedback loop

Against this background, a recent observation takes on special interest. Costa and his coworkers (74) have shown that exposure of carotid body to hypoxia (twice for 30 min) causes a long-term induction of tyrosine hydroxylase (the rate-limiting enzyme of catecholamine synthesis) that requires protein synthesis. This induction of tyrosine hydroxylase fails to occur if the CSN is trans-sected, thus representing “trans-synaptic” enzyme induction.

This observation implies that feedback loops, probably reciprocal synaptic contacts between type IA cells and afferent nerve endings, are important in mediating transsynaptic enzyme induction during hypoxia. It appears possible that during hypoxia a chemical message is released from the afferent nerve endings at the efferent synaptic contacts and that this message is involved in tyrosine hydroxylase induction.

Because depolarization of afferent nerve endings is necessary for the release of a chemical message, dopamine secretion by type IA cells has to be inhibited previously. Perhaps this inhibition of type IA cells during hypoxia and concomitant transsynaptic enzyme induction is the reason that the rate of transmitter synthesis exceeds the rate of transmitter release.

A published electron micrograph of the carotid body in chronically hypoxic animals (75) is strikingly in line with this idea: one glomus cell with only few and small dense-cored vesicles is presynaptic to another glomus cell with abundant large dense-cored vesicles.

In conclusion, we propose that information in the carotid body (type IB cell–type IA cell–afferent nerve ending–type IA cell) is processed in a fashion graded rather than digital, providing a finely adjusted and modulated cooperation of all elements.

On the basis of our present knowledge, it seems that our hypothesis represents only a further addition to a bewildering variety of hypotheses on chemoreceptor function. But it integrates everything presently known about structural units and synaptic interconnections of the carotid body parenchyma.

I thank Prof. Dr. P. Heistracher and Drs. M. Lischka and J. Graf for constructive criticism and for the invitation to prove this hypothesis. I also thank Dr. C. Pernecky for critically reading the manuscript.
