Innervation of the Chief Cells of the Carotid Body: An Ultrastructural Review

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Summary. Recent serial ultrathin section studies have shown that a single kind of nerve fiber innervates chief cells of the carotid body through various types of endings. These nerve endings have been described in previous papers as being vesicle-rich, mitochondria-rich or small and large calyciform; these endings occurred in en passant and bouton forms. The intracranial section experiments of the glossopharyngeal nerve seem to support the view that the nerve fiber innervating chief cells is sensory, the soma of which is located in the inferior (petrosal) ganglion, although conflicting data still exist. Two types of synapses have been found at the sites of apposition between nerve fibers and chief cells: one, named efferent or type 1 synapse, in which chief cells are postsynaptic; the other, named afferent or type 2 synapse, in which chief cells are presynaptic. The serial ultrathin section study has shown that a single nerve fiber forms both types, with the latter type predominating. The possibility is strongly suggested that most of efferent synapses on the chief cells are formed by sensory fibers which form numerous afferent synapses, but not by proper efferent fibers. The similarity between chief cells and SIF cells in the autonomic ganglia is suggested in terms of synaptic relations with neuronal elements.

Electron microscopic studies have revealed that the parenchyma of the carotid body consists of two distinct types of cells: chief (glomus type I) cells and sustentacular (glomus type II) cells. Chief cells contain many granular vesicles while sustentacular cell enclose the chief cells with their slender cytoplasm. Both are assembled into lobules. The carotid body is well vascularized with fenestrated capillaries and richly supplied with nerve fibers. The majority of nerve fibers are unmyelinated, while a few are myelinated. Each nerve fiber loses its Schwann sheath and external lamina when penetrating into lobule. In the lobule, it is directly ensheathed by sustentacular cells and eventually comes into direct apposition to chief cells with a narrow intercellular space, about 15 nm in width (for references, see Kobayashi, 1971).

Although the carotid body has been generally regarded as a chemoreceptor organ since the original work of De Castro (1928), there has been much controversy on the mechanism underlying its chemosensory function. Some considered that the chief cells are chemoreceptor cells and that the nerve fibers terminating on the chief cells are basically sensory, thereby participating in the conduction of chemosensory impulses (Eyzaguirre et al., 1972). On the other hand, the presence of microvesicles in the nerve fibers apposed to chief cells led others to the view that the nerve fibers are efferent to chief cells rather than afferent. They claimed that the chief cells are secretory or effector cells (Pearse, 1969; Bisceo et al., 1970; Tramezzani et al., 1971) and that the sensor of chemoreception should be a different identity from the chief cell (Bisceo et al., 1970).

Several articles have reviewed physiological and morphological aspects of this organ in some detail (Adams, 1958; Bisceo, 1971, 1974; Torrance, 1968; Purves, 1974).
The scope of this review is, therefore, confined to the ultrastructural analysis of nerve fibers apposed to the chief cells, since this analysis is crucial for a correct understanding of the function of the carotid body.

1. Vesicle-containing nerve fibers

Nerve fibers in the lobules contain small clear vesicles 50 nm in mean diameter, a few large granular vesicles 100 nm in mean diameter, small mitochondria, glycogen, neurofilaments and neurotubules. These elements, by electron microscopy, are very much alike among the different types of nerve endings. AL-LAMI and MURRAY (1968) distinguished two types which were named bulbous and basket endings. On the basis of the preponderance of a particular set of intracytoplasmic constituents, mitochondria-rich nerve endings were described as an entity different from vesicle-containing nerve endings (Böck et al., 1970; KONDO, 1971; Verna, 1971). Most of these studies, however, were based on electron microscopic observations of random sections; thus, it is possible that different features of neuronal profiles in random sections merely represent different planes of sections. It is, therefore, important to apply serial section analyses to the study of the innervation of chief cells.

NISHI and STENSAAS (1974) reconstructed many nerve endings on the chief cells of the cat carotid body with serial ultrathin sections and classified them into two types—large and small calyciform endings. KONDO (1976b) attempted a long serial ultrathin section study on the innervation of chief cells of the rat carotid body and completed the reconstruction of all nerve fibers in one lobule. It was revealed that a single branching nerve fiber comes into contact with many chief cells. It apparently formed various kinds of terminals, so far described previously using the terminology (vesicle-containing, mitochondria-rich large and small calyciform). The nerve endings occurred in en passant and bouton forms. It was verified that different features of neuronal profiles in random sections are ascribed to different planes of sections. The author concluded on the basis of this observation that the chief cells are principally innervated by a single kind of nerve fiber. A similar conclusion was reached by Verna (1973) in a random section study of the rabbit and by BISCOE and PALLOT (1972) in a short report of the cat carotid body with serial sections.

2. Degeneration study on nerve fibers apposed to the chief cells

There is a good deal of evidence that the severance of the sinus nerve of the glossopharyngeal nerve results in degeneration of nerve endings on the chief cells (Battaglia, 1966; Biscoe and Stehbens, 1966; Hess, 1968; Hess and Zapata, 1972; Nishi and Stensaas, 1974; McDonald and Mitchell, 1975). The majority of degenerative changes occurs in the first 7 day after severance of the sinus nerve, although degeneration is not uniform among nerve endings (Hess, 1968; Abbott et al., 1972). The nerve endings become increasingly difficult to identify within the first month of the postoperative period (Biscoe and Stehbens, 1966). It is, therefore, most likely that all nerve fibers terminating on the chief cells, with various forms of nerve endings, are of glossopharyngeal origin.

Degeneration experiments have also been applied to solve the question whether the nerve fibers terminating on the chief cells are sensory or motor. Extracranial severance of the glossopharyngeal nerve distal to the inferior (petrosal) ganglion should result in degeneration of both motor and sensory fibers in this nerve. On the
other hand, intracranial severance of the glossopharyngeal nerve proximal to its ganglion should produce degeneration of motor fibers, while sensory fibers should remain intact after operation. Several authors experimented on intracranial severance of the glossopharyngeal nerve and provided conflicting results. De Castro and Rubino (1968) reported that 30 days after severance of the nerve, nerve endings on the chief cells were unchanged and still contained groups of mitochondria and synaptic vesicles. This experimental study confirmed De Castro's previous view, based on light microscopy, that the nerve fibers terminating on chief cells are sensory (De Castro, 1928). Biscoe et al. (1970), on the other hand, reported that after the operation, there was an absolute progressive reduction in the number of vesicle-containing nerve endings on the chief cells as compared with control non-operated side. They concluded that nerve fibers terminating on the chief cells are all motor; sensory endings participating in chemoreception might be situated in the interstitial space, not in contact with the chief cells.

Since this report, four papers have been published on intracranial severance experiments, in which a conclusion contrary to the one by Biscoe et al. was drawn (Hess, 1968; Hess and Zapata, 1972; Nishi and Stensaas, 1974; McDonald and Mitchell, 1975). In the experiment by Hess and Zapata, no changes in nerve fibers, either myelinated or unmyelinated near the glomus, or in the sustentacular cells, were detected after 75 days. Nishi and Stensaas also reported that most nerve endings in the carotid bodies of cats which survived up to 21 days after intracranial severance remained unchanged. They concluded that the nerve fibers terminating on the chief cells were sensory.

Hess and Zapata conducted the following studies in order to test whether or not the intracranial severance experiment was successfully performed: They examined, histologically and physiologically, degeneration of nerve fibers in taste buds of the posterior third of the tongue which are innervated by the sensory components of the glossopharyngeal nerve and also nerve fibers in the superior pharyngeal constrictor muscle which are innervated by the motor components of the glossopharyngeal nerve. These additional tests seem to have confirmed the reliability of their results. They attempted to give an interpretation upon the observations of Biscoe et al. by pointing out the possibility that the intracranial section of the glossopharyngeal nerve performed by Biscoe et al. might be so close to the petrosal ganglion that it caused injury or retrograde degeneration of the ganglion cells, which led to the degeneration of distal processes of the ganglion cells and synapses on the chief cells.

3. Synapses on the chief cells

By electron microscopy, the chemical synapses have been recognized as sites where clusters of small vesicles are in close association with the cytoplasmic surface of the presynaptic membrane. At such sites, the presynaptic membranes lie parallel to each other with a gap of approximately 20 nm. The cytoplasmic surface of one or both membranes is coated with electron dense material. This coating is either symmetric. In cases where neuronal profiles on both sides contain vesicles, the profile with a vesicle accumulation close to the synaptic membrane is considered to be the presynaptic component (Pappas and Purrura, 1972; Peter et al., 1976).

It should be noted that most electron micrographs so far published in previous studies of the carotid body presented only junctional sites with a symmetric coating.
of dense material similar to the zonula adherens. At these sites, no clustered vesicles were seen close to the junctional membrane though many vesicles were scattered in the neuronal profile. Only a few reports showed typical synapses fulfilling the morphological criteria for chemical synapses as described above (Blümcke et al., 1967; Kobayashi, 1969; Buck et al., 1970; Knoche et al., 1971). Recent studies on the central nervous system have revealed the presence of axo-axonic and dendro-dendritic synapses in which neuronal profiles containing many scattered vesicles are postsynaptic (for references, see Peters et al., 1976). It is, therefore, necessary to re-investigate the relation between neuronal profiles and the chief cells in light of the present criteria for chemical synapses.

In this regard, two types of synapses have been recently identified in the carotid bodies of rodents including rats (King et al., 1975; McDonald and Mitchell, 1975; Morgan et al., 1975; Kondo, 1976b), mice (Kobayashi and Uehara, 1970), rabbits (Verna, 1973), and of birds (Kobayashi, 1971; Osborne and Butler, 1975). In one type the neuronal profile is considered to be presynaptic (efferent synapses), and in the other to be postsynaptic (afferent synapses). The former type of synapse is characterized by a more marked accumulation of electron dense material on the side of the neuronal profile than on the chief cell side. A cluster of vesicles, 50 nm in diameter, is closely associated with the accumulation of dense material, whereas no clustered vesicles are found on the chief cell side. The latter type is characterized by electron dense material accumulated mainly on the chief cell side, where it forms a series of short projections (presynaptic projection). Large granular vesicles characteristic of the chief cell and small clear vesicles, 50 nm in diameter, are concentrated close to the dense material. The apposed membrane of neuronal profile is slightly thickened, but no vesicles are clustered in the subjacent axoplasm. Large granular vesicles are frequently seen lying in more than one row.

In the mouse carotid body, some small vesicles inside the chief cell contain a dense core (Kobayashi and Uehara, 1970), while in the rat and rabbit, small dense cored vesicles are rarely found. McDonald and Mitchell (1975) reported that after treatment with 5-OHDA, dense precipitates appeared in nearly 40% of the small clear vesicles in chief cell of the rat carotid body. On the contrary, the present author has failed to notice any precipitates using the same experiment (unpublished data). Thus, the functional significance of these small vesicles at the synaptic junction still remains uncertain.

Developmental studies of the rat carotid body (Kondo, 1975, 1976a) revealed that these two types of synapses first occur in a 17 mm CR (crown-rump) length embryo. In these studies the efferent type of synapse is named type 1, and the afferent is type 2; the number of synapses per unit area (60,000 μm²) was counted throughout the entire course of development. As development proceeds, type 2 synapses rapidly increase in number, while type 1 synapses increase in number very little. Eventually in 50 days old rats, type 2 synapses are approximately 22 times more numerous than type 1.

A three dimensional reconstruction study of nerve fibers in the rat carotid body with long serial ultrathin sections (Kondo, 1976b) revealed that a single branching nerve fiber forms many synapses of both types, predominantly of type 2, with a number of chief cells. The author concluded that the main direction of transmission is from chief cells to nerve fibers, and hence the nerve fiber is basically sensory.
This conclusion runs counter to the general view that nerve endings with numerous small vesicles are motor (efferent) in nature (PAPPAS and PURPURA, 1972). The presence of an unexpectedly large number of vesicles in the presumed sensory nerve fiber, in spite of the small number of type 1 synapses formed by this fiber, leads us to ask the following questions: What is the function of the vesicles scattered in the presumed sensory nerve fibers? Does the number of these two types of synapses vary depending on the conditions of fixation? The solution to these problems may lay in the comparative study of various species by means of long, serial sections.

4. Do true motor (efferent) nerve fibers terminate on the chief cell?

Although many papers reported that true efferent fibers terminate on the chief cells, most of them did not show any synapses that fulfill the present morphological criteria for efferent synapses as mentioned in the previous section. Since the serial

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Fig. 1. Postganglionic sympathetic (adrenergic) nerve fibers (A) enclosed together by common sustentacular cell (S) with chief cells (C) of the rat carotid body. Note small dense cored vesicles in the nerve fibers. Two hrs after the injection of 5-OHDA (100 mg/kg). x 20,000

Fig. 2. A synapse formed by adrenergic nerve fiber (A) on chief cell (C) of the rat carotid body. Two hrs after the injection of 5-OHDA (100 mg/kg). x 30,000

Fig. 3. Small dense cored vesicles in the perikarya of ganglion cells of the rat carotid body. Two hrs after the injection of 5-OHDA (100 mg/kg). x 40,000

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ultrathin section analysis revealed that efferent synapses on the chief cells are formed by the nerve fiber which predominantly forms afferent synapses on the same cells, it may be premature, without serial section analysis, to conclude the presence of true efferent nerve fibers in addition to these afferent ones. Several authors described efferent nerve fibers terminating on sensory nerve fibers (Kobayashi, 1969; Verna, 1973) in random section studies. However, it should be noted that, unless the serial section analysis is employed, it is almost impossible to distinguish without fail between neuronal profiles and slender processes of the chief cells. It is highly probable that the efferent nerve fibers so called by these authors are, in reality, processes from chief cells.

In their degeneration experiment, McDonald and Mitchell (1975) reported that 5% of total nerve endings on the chief cells derive from preganglionic sympathetic axons. Kondo (1976b) noted that preganglionic axons which terminate on the ganglion cell form efferent synapses also with the chief cell.

In addition, a few postganglionic sympathetic (adrenergic) nerve endings containing small dense cored vesicles make synaptic contacts with chief cells of the rat carotid body (Fig. 1, 2). The incidence of this type of nerve endings is 2/1000 in ratio (number of sympathetic nerve endings on chief cells to number of chief cells in single section) (McDonald and Mitchell, 1975).

5. Ganglion cells in the carotid body

Although the presence of a few ganglion cells in the carotid body has been well documented by light microscopic studies, there have been only two reports describing the detailed structure of the ganglion cells as revealed by electron microscopy (McDonald and Mitchell, 1975; Kondo, 1976b). In their degeneration experiment McDonald and Mitchell (1975) reported that most of the ganglion cells in the rat carotid body are parasympathetic and are innervated by neurons in the brain, axons of which travel through the glossopharyngeal and sinus nerve. They also noted a few sympathetic ganglion cells which are innervated by preganglionic nerves from the sympathetic trunk. Ganglion cells containing small dense cored vesicles, 50 nm in mean diameter, in their perikaryon are found in the rat carotid body after the administration of 5-OHDA (Fig. 3). Kondo (1976b) described chief cells located in the periphery of this organ which are in synaptic relation with dendrites of the intrinsic ganglion cells, where the chief cells are presynaptic.

Conclusion

There have been a number of physiological and pharmacological studies on the carotid body, and many hypotheses have been suggested to explain its chemoreception mechanism (Purves, 1974; Osborne and Butler, 1975). However, these hypotheses have remained largely unverified. As shown in this review, nerve endings distributed in this organ are complicated in structure. The presence of reciprocal synapses between chief cells and nerve fibers and synaptic contacts between adjacent chief cells are noted (McDonald and Mitchell, 1975; Morgan et al., 1975; Kondo, 1976b). The serial section study (Kondo, 1976b) implies that some chief cells are innervated by two or more sensory nerve fibers. Although the presently available data described above (summarized in Fig. 4) favor the hypothesis that the chief cells are chemosensory cells whose excitation is transmitted to afferent endings of the sinus
nerve, mechanisms more complicated than those so far suggested for the origin of chemosensory discharges should be proposed for consideration.

Cells with ultrastructural characteristics similar to those of the chief cell have been reported in various autonomic ganglia, particularly in the superior cervical ganglion of rats and they are commonly named SIF (small intensely fluorescent) cells (CHIBA, 1977). It is generally considered that SIF cells could be interposed as interneurons between preganglionic and postganglionic neurons. However, this interpretation is not fully confirmed by experimental findings (MATTHEWS and OSTBERG, 1973). The serial ultrathin section study on the carotid body (KONDO, 1976b) presented a new finding that vesicle-containing nerve endings are sensory, which runs counter to the hitherto accepted view. Therefore, laborious serial ultrathin section analysis should be made on the innervation of the SIF cells, to determine whether or not the synaptic connections of SIF cells with neuronal elements are similar to those of chief cells in the carotid body and to re-examine the assumption that SIF cells may act as interneurons in ganglionic transmission.

Fig. 4. Diagram of synaptic connections of chief cells (C) with neuronal elements in the rat carotid body. Arrows indicate presumed direction of nerve conduction and synaptic transmission. Ad postganglionic sympathetic (adrenergic) nerve fiber, E endothelial cell of fenestrated capillary, G ganglion cell.

頸動脈小体主細胞の神経支配に関する電子顕微鏡による研究の総説

近藤 善武

頸動脈小体の超薄連続切片による観察の結果，これまで記載されてきた主細胞に終わる種々の型の神経終末（多量の小胞を含む終末，多量の糸粒体を含む終末，大型お


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