Occurrence of Afferent Synaptic Complexes in the Carotid Body of the Mouse

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Physiological studies have shown that the carotid body of various mammals such as the cat and dog is a chemoreceptor (Heymans and Neil, 1958; Comroe, 1964; Torrance, 1968), and the chief or glomus cells of this organ have long been supposed to be receptor cells. Electron microscopic studies have revealed many nerve terminals around the chief cells, partly in direct contact with the latter. Physiologists have believed the nerve terminals to be of an afferent nature, but ultrastructural findings to support this belief have not yet been found; previous findings indicating the occurrence of synaptic vesicles only within the nerve terminals and not in the chief cells have recently compelled some electron microscopists, including one of the present authors, to assume that the nerves on the chief cell might be of efferent nature (Biscoe and Sterbens, 1966; Abraham, 1968; Kobayashi, 1968).

In this study, we examined under the electron microscope the carotid body of the normal mouse, with special reference to the discrepancy between the interpretations of this organ by physiologists and by morphologists.

Methods

Nine adult albino mice weighing 20–30 g were used. Animals anesthetized by an intraperitoneal injection of sodium pentobarbital solution were perfused with 2.5% glutaraldehyde buffered with 0.09 M phosphate at pH 7.1 from the left ventricle by means of a thin polyethylene tube. The carotid bodies of both sides together with surrounding tissues were taken and further fixed for several hours in the same glutaraldehyde solution followed by OsO₄ post-fixation. The fixed tissues were dehydrated with a graded series of ethanol, treated with propylene oxide and embedded in Luft's Epon. Thin sections were made by glass knives on a Porter-Blum microtome and stained doubly with uranyl acetate and Millonig's lead. Observations were performed using the Hitachi HS 7s and HU 11ds electron microscopes.

Observations

The carotid body of the mouse was present at the carotid bifurcation, on the medial side of the vessels. It was closely attached to and sometimes even in direct contact with the superior cervical ganglion and consisted of several nests of chief or glomus cells which were enmeshed in tangles of twining nerve fibers and blood capillaries. Thus, the position and the histology of the carotid body of the mouse were comparable to those of other mammals such as the cat and dog (see Adams, 1958).
Fig. 1. A low-power electron micrograph of the mouse carotid body showing a nest of several chief cells (C) containing numerous dense specific granules. Schwann cells (S) and nerve processes or terminals (n). The endothelium of the blood capillary (E) is fairly fenestrated. F fibroblast. ×6,000
The chief cells of the mouse like those of other mammals, were incompletely enveloped by Schwann or sustentacular cells and were characterized by a number of membrane-bound cytoplasmic granules, 80-90μm in diameter, corresponding in fine structure to the catecholamine-containing granules of the adrenal medullary cell. Observations on the cell organelles such as the Golgi complex, centrioles and flagellum, mitochondria, lysosomes, endoplasmic reticulum, cytoplasmic filaments and microtubules, essentially corresponded to those of other mammals as reported previously by Lever, Lewis and Boyd (1959), Battaglia (1968), Grimley and Glenner (1968), Kobayashi (1968) and others.

Abundant profiles of nerve terminals found in the space between the chief cell and the Schwann cell (Fig. 1). They were identified by the presence of regularly aligned microtubules, small mitochondria and different types of microvesicles. The most commonly occurring microvesicles were about 40μm in diameter, roughly spherical in shape and showed no apparent contents. Small numbers of larger

![Image](image-url)

**Fig 2.** Carotid body of the mouse. There are three afferent synaptic complexes (S1, S2, S3) formed between a chief cell and two profiles of nerve terminals containing a few mitochondria and synaptic vesicles. At the right-lower corner a portion of a nucleus (N) is seen. **n** non-myelinated nerve process, **p** process of a Schwann cell. ×40,000
dense-cored granules, 70–90 nm in diameter, were mixed with them (Fig. 2, 3, 4).

Desmosome-like structures were frequent between nerve terminals and the chief cell. Here the distance between the apposed plasma membranes was rather constant and measured about 25 nm in width. The dense, filamentous material attached to the junctional membrane was somewhat more prominent on the chief cell side, where it resembled pre-synaptic dense projections (Gray and Guillery, 1966) (Fig. 4). On the nervous side the microvesicles were usually located away from the junctional zone, while on the chief cell side there was an accumulation of small cored vesicles. These were usually spherical in shape, about 30–40 nm in diameter, surrounded by a single membrane sac and filled with an electron-dense, granular substance (Fig. 2, 3, 4). Occasionally several assemblages of small cored vesicles plus desmosome-like structures between a chief cell and a nerve terminal were found (Fig. 2). There seemed to be no transitional forms between these small cored vesicles and the specific granules mentioned above.

Not every desmosome-like structure formed between the nerve terminal and the chief cell was associated with accumulations of microvesicles. When the latter were
absent both on the nervous side or chief cell side, they were considered to be purely adhesive and to deserve the name puncta adherentes (FARQUHAR and PALADE, 1963; PETERS, PALAY and WEBSTER, 1970) (Fig. 4, 5).

**Discussion**

The distribution of biogenic monoamines in the tissues has recently come to be studied using the fluorescence microscopical techniques developed by ERÄNGÖ, FALCK and HILLARP (DAHLSTRÖM and FUXE, 1965; CORRODI and JONSSON, 1967). It has thus become evident that the chief or glomus cells of the carotid body, without a single exception, contain catecholamines (RAHN, 1961; NIEMI and OJARA, 1964; BLÜMCKE, RODE and NIEDORF, 1967; DEARNALEY, FILLENZ and WOODS 1968; UEHARA in press). Nor has an exception been found to the generalization that the chief cells of the carotid body contain more or less numerous specific dense granules first described in 1957 by LEVER and BOYD (LEVER, LEWIS and BOYD, 1959; BISCOE and STEHBENS, 1966; HESS, 1968; KOBAYASHI, 1968). The resemblance of the latter to catecholamine-containing granules of the adrenal medullary cells was interpreted to indicate that catecholamines in the carotid body might be contained in these granules. There have been a few authors who regarded the chief cell granules as concerned with the afferent innervation (LEVER, LEWIS and BOYD, 1959; IISHI and IISHII, 1967; GRIMLEY and GLENNER, 1968). However, these granules are not always aggregated in the areas where the nerve terminals are in apposition. Furthermore, figures suggesting emiocytotic release of the granules were observed not only in the innervation area of the chief cell but also in the free cell surface and in the surface areas facing the adjacent chief cells (BISCOE and STEHBENS, 1966; BLÜMCKE, RODE and NIEDORF, 1967; KOBAYASHI, 1968; UEHARA in press). These facts seem to suggest that the content of the specific granules, which is presumed to be catecholamines, represents a hormone rather than a neurotransmitter; thus, the present authors can not agree with the opinion that the specific granules are involved in the generation of the chemosensory discharge.

It seems to be now generally accepted that all of the chemical synapses in the central nervous system so far studied consist of (1) a presynaptic element containing accumulations of synaptic vesicles, (2) a post-synaptic element, and (3) a synaptic cleft 20–40mμ wide; the pre- and post-synaptic membranes come into apposition with dark, filamentous material condensed in the adjacent cytoplasm to form a kind of macula adherens (ECCLES, 1964; GRAY, 1969). PALAY (1958) referred to the whole assemblage of vesicles plus membrane thickenings as a synaptic complex. It is believed that the aggregation of vesicles in the synaptic complexes suggests that they may be sites for the extrusion of the chemical transmitter into the synaptic cleft (GRAY and GUILLERY, 1966; PETERS, PALAY and WEBSTER, 1970).

The assemblages of small cored vesicles and desmosome-like structures observed in this study appear identical with synaptic complexes in the central nervous system. They are apparently afferent if judged on the basis of the position of the vesicles. The occurrence of microvesicles, if not numerous, on the nervous side may be unimportant in hindering identification of the nerve terminals as afferent because it is known that some sensory nerve endings such as those in the taste bud (MURRY and MURRY, 1967; UGA, 1969) and inner ear (SMITH and SJÖSTRAND, 1961; FLOCK, 1964, WERSÅLL, 1968) may contain a few synaptic vesicles.
The occurrence of afferent type synaptic complexes does not prove, but only suggests, a possible receptor function of the chief cell. As far as the authors know, there is no report on the physiology of the mouse carotid body and it remains obscure whether the chief cell is really a chemoreceptor in this animal. Therefore, physiological studies in this animal are desired for the correlation of the morphology and the function of the carotid body.

Although the ultrastructure of the small cored vesicles observed in this study is similar to those which were considered to contain catecholamines (Richardson, 1962; Wolfe, Potter, Richardson and Axelrod, 1962; Richardson, 1964), no direct evidence is available on this point. Heymans and his co-workers suggested that acetylcholine might be a sensory transmitter in the carotid body (Heymans and Neil, 1958) and this proposition was later supported by the studies of Eyzaguirre and his co-workers (Eyzaguirre and Koyano, 1965; Eyzaguirre, Koyano and Taylor, 1965; Zapata et al., 1969). On the other hand, there are opinions that catecholamines may play an important role in the generation of chemosensory discharges in the carotid body (Ishii and Ishii, 1967; Grimley and Glenn, 1968). Studies on the chemical nature of the content of the small cored vesicles are desired, as this may lead to the elucidation of a chemosensory transmitter, if present.

The present paper makes clear the need for re-examination of the ultrastructure of the nerve terminals in the carotid body of various mammals such as the cat and dog where vesicular packets were found on the "wrong" side; that is, they were found only on the nervous side of the synaptic complex (Biscoe and Stehbens, 1966; Ábrahám, 1968; Hess, 1968; Kobayashi, 1968). It may be worthy to add lastly that, after the finding of afferent synaptic complexes in the mouse carotid body, the authors tried to discover whether a similar structure was present in the carotid body of a 15 week human fetus. However, such a structure has not yet been found, though typical efferent synaptic complexes were frequently observed as shown in Figure 5.

Summary

The carotid body of the mouse was studied under the electron microscope. The chief cell contained numerous specific dense granules, 80–90 mμ in diameter, resembling catecholamine-containing granules of the adrenal medullary cells.

Characteristic small cored vesicles about 30–40 mμ in diameter were found within the cytoplasm of the chief cell. These vesicles aggregated particularly in the region where the nerve terminals were in apposition. Assemblages of small cored vesicles and desmosome-like structures between the chief cell and the nerve terminal were comparable to synaptic complexes described in the central nervous system. On the basis of the localization of the small cored vesicles these synaptic complexes were apparently afferent.

If the chief cell of the mouse carotid body is really a chemoreceptor as is thought by physiologists, it is highly probable that the synaptic complexes described in this paper correspond to the site of sensory transmission.
マウスの顕動脈小体にみられた 求心性シナプスと思われる構造
（内容自抄）

電子顕微鏡下に マウスの顕動脈小体を 観察した．主細胞は 副腎髄質のカテコールアミンを含む顆粒に似た 直径 80〜90mμ の暗調な顆粒を 多数 含んでいた．
主細胞の細胞質に 従来記載のない 小型の芯あり小胞が 見出された．この小胞は 直径約 30〜40mμ で，主細胞と神経終末が接している部分に限局して みられた．小型芯あり小胞の集合と 神経終末と主細胞との間に形成された接着膜との組み合わせは 中枢神経系で シナプスの形態的示標とされている構造に酷似しており，小型芯あり小胞の集合が主細胞側にあることより 求心性のシナプスに相当するであろうと考えられた．

マウスの顕動脈小体の主細胞が 化学受容細胞であることが 生理学的に証明されるならば，本研究で明らかにされた 小型芯あり小胞と接着膜の組み合わせが 感覚性の興奮伝達の場所であろう．

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