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THE STRUCTURE AND CATECHOLAMINE CONTENT OF THE

CAROTID BODY

A THESIS PRESENTED BY

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FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
THE UNIVERSITY OF LEICESTER

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SEPTEMBER 1983

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A B S T R A C T

The thesis reviews previous work on the ultrastructure and catecholamine content of the carotid body and describes areas of sparse available data.

- 1) The question of sub-populations of Type I cells on the basis of quantitative ultrastructure;
- 2) The reaction of the mitochondria and electron dense-cored vesicles of the Type I cells to physiological hypoxic stimuli;
- 3) The levels and type of catecholamines, stored within the carotid body;
- 4) The influence of sympathectomy on these levels;
- 5) The influence of hypoxia, hyperoxia and hypercapnia on catecholamine levels.

The experimental data obtained has revealed that the rat carotid body contains only one cell population, whilst that of the cat contains two. Both organs show ultrastructural changes in the Type I cells in response to moderate hypoxia.

The catecholamine content studies have shown that the rat stores predominantly dopamine in its Type I cells. In confirmation of previous studies hypoxia is shown to deplete dopamine levels; a new and potentially important finding is that

hypercapnia and hyperoxia increase dopamine levels.

The possible significance of these findings is discussed and a testable hypotheses suggested.

CHAPTER ONE

CHAPTER 1

REVIEW OF THE LITERATURE

1.1. EARLY STUDIES

According to Valentin (1833) and Luschka (1862), the first description of the carotid body must be ascribed to a Swiss physiologist - Albercht von Haller - who, in 1762, mentioned it as the "ganglion exiguum".

There exists, however, several more accurate descriptions before 1762. For example Taube (1743), a student of von Haller, described the carotid body as the "ganglion minutum". Again Andersch in 1797 reprinted the work of his father, which he claimed was performed between 1751 and 1755. The original printing of this work was sold as wastepaper. Andersch called the organ the "gangliolum intercaroticum", due to its location; he also states specifically that the sympathetic, the glossopharyngeal and the vagus nerves sent branches into the organ.

For a while the carotid body remained forgotten, to be "newly" discovered by Mayer of Bonn in 1833, who recognised branches of the sympathetic, glossopharyngeal and vagus nerve as the sources of the nerve plexus which innervated the "ganglion intercaroticum". Valentin (1833) regarded the structure^{as} part of the sympathetic nervous system although he too recognised that the vagus and glossopharyngeal contributed conspicuously to its

innervation.

Thus, it becomes evident that the anatomists of the eighteenth and early nineteenth centuries regarded the little structure in the carotid bifurcation as one of the many ganglia which are interspersed in the course of the sympathetic. The carotid body received little attention for the next thirty years, indeed until microscopic investigation became more fashionable. Beginning with Luschka (1862) the search for its microscopical nature touched off a controversy which lasted until recent years.

Luschka (1862) revealed that the organ was not a ganglion but a glandular structure rich in nerves and blood vessels; thinking it was derived from endoderm, he named it the "glandula carotica". Luschka was hence the first to break with the tradition of interpreting the little structure as another sympathetic ganglion; he was undoubtedly the first to recognise the glandular character of this organ and, by recognising its morphological kinship with the adrenal and pituitary gland, started one of the lines of thought which have continued to the present day.

Arnold (1865) rejected Luschka's interpretation and insisted that the structure was nothing more than a vascular glomerulus; he named it "glomeruli arteriosi intercarotici", and considered that the chief cells in the carotid body were derived from the endothelial cells of the blood vessels. Considerable controversy soon arose between the adherents to Arnold's vascular hypothesis and those who followed Luschka's interpretation.

About this time the field of embryology was gaining increasing importance. It was Kolliker in 1879 who first suggested the study of the development of the carotid body. Stieda (1881) regarded a thickening of the epithelium of the third branchial cleft as the anlage of the carotid body since this proliferation is placed closely to the carotid bifurcation. Prenant (1894) concurred with Stieda (1881), whilst Born (1883) and Prenant (1899) in a later publication doubted the branchial origin of the "carotid body".

In 1887 Kastschenko declared that "this structure is not a derivative of the branchial cleft but develops from a proliferation of the wall of the internal carotid artery. Subsequently the glossopharyngeal CN (N IX), vagus CN (N X) and the cervical sympathetic make contact with the organ". Similar contacts were described by Marchand (1891) Paultauf (1892) and Verdun (1898). In addition, Marchand (1891) added another name to the literature: he called the carotid body "nodulus caroticus".

In 1894 Prenant claimed the carotid body was derived from the third branchial cleft and temporarily joined the thymus primordium; he called it "glanduli thymique". Jacoby (1896) showed Prenant to be wrong in that what Prenant had demonstrated was in fact the primordia of the parathyroid III (described by Sandstrom in 1880) which, in some species, retains its position in the carotid fork. Jacoby concurred with Kastschenko that the carotid body is a derivative of the wall of the carotid artery.

Thus far, however, all these studies did not help much in comprehending the nature of this organ. The question still remained as to whether to classify it as a mere vascular glomerulus, a gland, part of the autonomic nervous system or a combination of these possibilities.

In 1898 Stilling first described a chromaffin reaction in the carotid body. Since Henle (1865), this reaction had been known in the adrenal medulla and was thought to be a specific technique for histochemical detection of adrenaline (and other amine-like substances). Subsequently, the chromaffinity or otherwise, of carotid body cells has been one of the most disputed questions in the study of the chemoreceptors. In 1903 Kohn defined the carotid body as being derived from neuro-ectoderm, innervated by preganglionic sympathetic nerve fibres and showing a positive chromaffin reaction; he considered the organ to be similar to the adrenal medulla and other paraganglia, hence another term "paraganglion intercaroticum". Although he called the chief cells of this tissue the "chromaffin cells" it is doubtful that Kohn himself recognised a chromaffin reaction in all of the cells of the carotid body. Kose (1907), one of the protagonists of Kohn's 'paraganglion' theory did not see a chromaffin reaction in these cells and, therefore, gave them the name "farblose chromaffin zellen" - clear chromaffin cells.

The carotid body received practically no attention for the next 30 years except for mentions in textbooks of histology under headings such as "endocrines", "chromaffin tissue" or

"paraganglion caroticum" and, in some English texts, under the non-committal name "carotid body".

The most outstanding contribution to the knowledge of the significance of the carotid body was made by de Castro of Madrid in the latter half of the 1920s. Between 1926 and 1928 he performed the first systematic studies of the innervation of the carotid body. Using methylene blue and silver techniques de Castro noted numerous nerve fibres and terminals in association with parenchymal cells and described the following features of carotid body nerve fibres (de Castro, 1926, 1928; and see Eyzaguirre & Gallego, 1975):

a) individual nerve fibres undergo branching to innervate many cells, often located in different glomeruli;

b) cells may be innervated by more than one nerve fibre;

c) a single nerve fibre may give rise to terminals displaying variable morphologies, including small boutons, cup-shaped (calyceal) endings and large plate-like endings and nerve terminals often give rise to fibres forming terminals contacting other cells;

d) nerve fibres and terminals in the carotid body are derived primarily from the carotid sinus nerve, (CSN a branch of the glossopharyngeal nerve (CN IX)) section of which produced degeneration of fibres and terminals within the carotid body.

De Castro (1928) sectioned the rootlets of CN IX

intracranially and carotid bodies were examined twelve days after surgery. He observed no degenerative changes and concluded that nerve endings on the cells were sensory with their cell bodies located in the petrosal ganglia. It was further postulated that Type I cells were chemoreceptors and that their sensory innervation was through the CSN (Herring's nerve) which de Castro called "intercarotidiene" because Herring's nomenclature did not define either the anatomical distribution (both to sinus and glomus) or the function of the nerve (presso-receptor and chemoreceptor pathways). Hence de Castro in his morphological studies firmly established the existence of two types of sensory receptors at the level of bifurcation of the common carotid artery, presso-receptors and chemoreceptors and, for the first time, the "glomus caroticum" was described as a sensory, independent organ. De Castro also proposed that the carotid body was not a paraganglion but a chemoreceptor which perceived the concentration of oxygen and carbon dioxide within the blood and introduced the term "pseudo-chromaffinity" to describe the brown colouration of part of the chief cells when treated with chromium salts which he thought was due, not to adrenaline-derived compounds, but to the reducing action of cytoplasmic lipid inclusions. De Castro ascribed polarity to the chief cells with one pole of the cell directed towards adjacent sinusoids (pole sanguin) and the opposite pole associated with nerve endings (pole nerveaux) (de Castro, 1940). The vascularity of the carotid body was examined in detail (de Castro, 1951) and numerous anastomosing blood vessels demonstrated.

The suggestions of de Castro were confirmed by

physiological experiments which showed that the area of the carotid bifurcation was a peripheral reflexogenic area sensitive to hypoxia and hypercapnia (Heymans & Heymans, 1927; Heymans, Boukaert & Regniers, 1933). The discovery of afferent nerve activity in whole CSN recordings which increased in response to asphyxia (Heymans & Rijlant, 1933) confirmed the belief that the carotid body was a sensory organ and supported the theories of de Castro.

During the 1930s when the chemoreceptor theory of de Castro was becoming popular, Kohn's paraganglion theory was essentially revised by his pupils, Watzka (1931; 1938; 1943) and Penitschka (1931). They classified the paraganglion into two categories, i.e. chromaffin paraganglia, originating from the sympathetic nervous system, and non-chromaffin paraganglia derived from the parasympathetic nervous system. According to them, the carotid body was the representative of the non-chromaffin paraganglia. Among the supporters of this theory were Palme (1934) and Seto (1935; 1949).

During the 1950s and 1960s the fluorescent methods for the histochemical demonstration of biogenic monoamines appeared. Eranko's method (1951; 1952; 1954; 1955) of detecting amine-storing cells was applied to the study of the carotid body by Muscholl, Rahn and Watzka (1960), Rahn (1961), Niemi and Ojala (1964) and by Palkama (1965) in different species. They found that some cells of the carotid body showed specific fluorescence after fixation with formalin.

In the 1960s Falck and Hillarp improved Eranko's method by introducing freeze-drying and formaldehyde gas treatment. This method was more sensitive and afforded far better localisation of biogenic monoamines in various tissues than Eranko's method. Numerous studies with this histochemical technique established that the majority of carotid body cells in a number of species showed a distinct formaldehyde-induced fluorescence and hence it was concluded that the carotid body must contain amines (see Biscoe, 1971, for review).

1.2. EMBRYOLOGICAL ORIGIN

At the end of the last century and the beginning of this, investigations on the origin of the carotid body were based upon the classical technique of descriptive embryology and produced controversial results (see Pearse, 1973).

Early in the twentieth century the popular view was that the carotid body chief cell was derived from cells that differentiated from sympathetic neuroblasts and were identical with the cells of the adrenal medulla and associated paraganglia (see Boyd, 1937).

Some authors (e.g. Kastchenko, 1887; Rabl, 1922; Smith, 1924; Rogers, 1965 Kondo, 1975) regarded the carotid body as a mesodermal derivative taking origin from a condensation of mesenchyme around the internal carotid artery. The CN IX, a branch of the vagus (CN X) and cervical sympathetic fibres, then

grew down to reach the forming carotid body primordium contributing various cellular elements to it (Smith, 1924; Boyd, 1937; Batten, 1960; Kondo, 1975).

It seems that the carotid body may have three sources for its cells:

- 1) neuroblasts migrating down the cranial nerves;
- 2) sympathoblasts from the superior cervical ganglion;
- 3) mesenchymal cells from the primary condensation on third aortic arch.

The early work of many authors utilised the chromaffin reaction. Some of these workers considered the carotid body as a derivative of the sympathetic nervous system because it showed some positive chromaffin reaction. The negative chromaffin reaction of many cells led other authors to assume that the cells of which the developing carotid body is composed were mesenchymal in origin (Korkala & Hervonen, 1973).

Using fluorescence techniques, Korkala & Hervonen (1973) showed that, in the seven week human fetus, three types of cells were observed in the surroundings of the carotid body. The primordium of the carotid body consisted of non-fluorescent fibroblasts and weakly fluorescent small cells with round nuclei. Similar cells were found in the sympathetic anlage and also in a cord of cells connecting the two structures. Among these two types of cells a third type of moderately fluorescent cell was

also seen. In the nine week fetus, the fluorescent intensity of the carotid body cells increased and the connecting cord of cells disappeared; at 11 to 16 weeks the carotid body was a separate entity with no cellular contacts to the sympathetic trunk or to the arterial mesenchyme - the fluorescent intensity reached its maximum in the twelfth week. The carotid body was fully mature with a separate fluorescent cell cord and highly organised vascularisation at between 17 and 22 weeks. In birds, at least, the study of Pearse et al. (1973) established that Type I cells of the carotid body arose from the neural crest.

Hence the carotid body seems to have a dual origin, a neural one which gives rise to the Type I cells and may be to the Type II cells and a mesenchymal one which gives rise to the remaining cells (fibroblasts, etc.) The best evidence suggests that the carotid body Type I cells are derived from the neural crest by the migration of a primitive sympathetic cell population along the nerves connecting the organ to the superior cervical ganglion and is hence embryologically similar to other paraganglia.

1.3. GROSS ANATOMY

The carotid body in the majority of animals is located somewhere near the bifurcation of the common carotid artery. In amphibians the carotid labyrinth, a swelling at the termination of each common carotid artery, is regarded as the homologue of the carotid body (Adams, 1958; Kobayashi, 1971a,b; Ishii &

Oosaki, 1966; Rogers, 1963). In birds the carotid body is found lateral to the common carotid artery in close proximity to the ultimo-branchial body (de Kock, 1958; Kobayashi, 1971a;) and is sometimes surrounded completely by parathyroid forming a parathyroid-carotid body complex (Kobayashi, 1969; 1971a).

In various mammalian species (e.g. man, cat, rat, camel, guinea pig, etc.), the carotid body is usually located at the carotid bifurcation and may be associated with any of the arteries arising from that general area - internal carotid, external carotid, occipital or ascending pharyngeal, depending upon species (de Kock, 1959, 1960; Lever et al., 1959; Ross, 1959; Al-Lami & Murray, 1968a; Grimley & Glenner, 1968; Kobayashi, 1968; Etemadi, 1975; Prakash & Rao, 1976; Morita et al., 1970).

1.4. STRUCTURE AND ULTRASTRUCTURE

1.4.1. LIGHT MICROSCOPY

A - Specific Cells

The mammalian carotid body is invested with a collagenous capsule which varies in thickness depending upon the species studied. The fundamental histological unit of the carotid body consists of clusters of two cell types which, together with an adjacent vessel, form a functional unit or glomoid. The principal cell type has been given different names over the years - amongst

them are 'chemoreceptor cell' (de Castro, 1926, 1951; Ross, 1959), 'specific cell' (Hoffman & Birreli, 1958), 'glomus cell' (Lever et al., 1959), 'enclosed cell' (Al-Lami & Murray, 1968a) and 'Type I cell' (Biscoe & Stehbens, 1966). The term 'Type I cell' is used throughout this thesis as it implies no particular functional attributes.

The Type I cells are arranged in small spherical or cylindrical anastomosing cords and are enmeshed in a skein of interlacing nerve fibres and fine capillary branches. The Type I cells are ovoid or polygonal in shape with a large round nucleus and are often separated from the capillary wall by processes of the second variety of cell - the Type II. In addition, the Type II cells partially surround groups of Type I cells. Various names have been given to the Type II cells, amongst which are 'sustentacular cell' (Ross, 1959; Grimley & Glenner, 1968), the 'pericyte cell' (Lever et al., 1959), 'enclosing cell' (Al-Lami & Murray, 1968a) and 'Type II cell' (Biscoe & Stehbens, 1966).

B - Blood Vessels

The carotid body is rich in blood vessels, most of which are typical capillaries, but a few are clearly fine branches of the arterial system, being large with thicker walls containing two or more layers of smooth muscle cells. The capillaries account for some 25% of total organ volume (Ballard et al., 1982).

The capillaries are very thin walled and surrounded by a

well developed basal lamina, each capillary is lined by two or more typical endothelial cells of variable thickness (Hodges et al., 1975). Under the electron microscope the endothelial cells have fenestrations (500 - 700 Å in diameter) (Biscoe & Stehbens, 1966) and exhibit many pinocytotic vesicles (Biscoe & Stehbens, 1966 ; de Kock & Dunn, 1966).

De Kock & Dunn (1964, 1966) claimed that in the cat the Type I cells are always surrounded and ensheathed by sustentacular cells and their processes; - thus Type I cells and capillaries are never in close proximity. This view has been challenged and is certainly untrue in other species (Hodges, et al., 1975; Verna, 1979).

In the camel, the Type I cells have direct contact with primary unit basement membrane and the basal surface of vascular endothelium and its associated basement membrane (Etemadi, 1975). This arrangement was also shown in the cat and rabbit by Biscoe and Stehbens (1966) and in rhesus monkey and cat (Al-Lami, Ph.D. thesis, 1964; de Kock, 1960; de Kock & Dunn, 1964, 1966; Al-Lami, 1964; Al-Lami & Murray, 1968a, 1968b; Biscoe & Stehbens, 1966; Morita, et al., 1970; Hodges et al., 1975; Bock et al., 1970; Etemadi, 1975).

C - Connective Tissue

Within the carotid body capsule large blood vessels and numerous myelinated and non-myelinated nerve fibres are present in the connective tissue stroma. The development of the

connective tissue is very variable in different species (Verna, 1979).

Within the connective tissue fibroblasts, plasma cells, eosinophils, collagen fibres, numerous mast cells and ganglion cells can be seen but no elastic fibres are present (Lever et al., 1959; Ross, 1959; Grimley & Glenner, 1968; Abraham, 1968; Al-Lami & Murray, 1968a, 1968b; Kobayashi, 1968, 1969, 1971a, 1971b; Morita et al., 1969, 1970; de Kock, 1959, 1960; Etemadi, 1975). The connective tissue stroma also contains groups of both myelinated and unmyelinated nerve fibres, the former being more common at the point of entry of the carotid sinus nerve. Nerve fibres, always by this time unmyelinated, enter the specific cell groups where they are enfolded in Type II cell cytoplasm.

1. 4. 2. ULTRASTRUCTURE

Whilst there may be quantitative differences in the carotid bodies of different species, the cell structure, as reported to date, is essentially similar in all species so far studied.

A - Type I cells

The Type I cells are complex in shape, with up to eight finger-like extensions projecting from the cell body; these processes are sometimes seen in close proximity to the vascular endothelium and basement membrane. The Type I cells are usually invested by Type II cells and associated processes except in

areas where they contact nerve endings, other Type I cells or are in close proximity to blood vessels (see Verna 1979,). The Type I cell contains a relatively large rounded nucleus which has a finely granulated appearance with a dense periphery and a single prominent nucleolus. Mitochondria are plentiful and evenly distributed in the cytoplasm of the cell body, whilst in the processes dense clusters or aggregations of mitochondria occur. A branched form of mitochondrion, in the process of dividing or budding, is not uncommon (de Kock & Dunn, 1966; Biscoe & Stehbens, 1966). The Golgi apparatus is sometimes very prominent and in a typical juxtannuclear position (Al-Lami, 1964).

The rough endoplasmic reticulum is well developed and frequently oriented in parallel lamellae (arrays) reminiscent of Nissl substance in neurons (Al-Lami, 1964). Microtubules, 170 - 250 Å in diameter - are prevalent, particularly in the centrosomal region and in close association with Golgi apparatus, aligned in parallel in Type I cell processes where organelles are sparse (Biscoe & Stehbens, 1966). Lysosome-like bodies are multilobed and often contain a rounded lighter zone of homogenous material (Al-Lami & Murray, 1968a, 1968b). Free grouped RNP (ribosomes) are frequently seen (Al-Lami, 1964) (Ross, 1959; Blumke et al., 1967; de Kock & Dunn, 1966; Al-Lami, 1964; Hoglund, 1967; Biscoe & Stehbens, 1966; Al-Lami & Murray, 1968a, 1968b; Grimley & Glenner, 1968; Kobayashi, 1968, 1969, 1971b; Kondo, 1971; Morgan et al., 1975).

Centrioles with their axes at right angles to each other have been described in Type I cells by several authors (see

Biscoe, 1971). Some of the centrioles were continuous with a cilium which may extend beyond the normal cell margin, has an overall diameter of approximately 2200 Å and circumferentially arranged fibrils around 250 Å each and together having a 9+1 or 8+1 pattern (Biscoe & Stehbens, 1966; Hess, 1968; Kondo, 1971; Morgan et al., 1975). Irregular dense bodies embedded in a fibrillar matrix which may represent a counterpart of lipid, fuchsenophil or lipofuscin pigment are frequently present in the cytoplasm (Al-Lami, 1964; Al-Lami & Murray, 1968a, 1968b).

Crystals with irregular bandings (spaces between bands range from 100 Å to 120 Å) were seen in the cytoplasm occasionally and in close association with lysosome-like dense bodies (Biscoe & Stehbens, 1966; Grimley & Glenner, 1966; Al-Lami & Murray, 1968a, 1968b).

Several authors described 'coated vesicles' of 400 - 1200 Å scattered throughout the cytoplasm (Biscoe & Stehbens, 1966; Hoglund, 1967). A junctional complex-like Zanulla adherence occurred occasionally between adjacent Type I cells (Biscoe & Stehbens, 1966).

The most striking and distinguishing feature of Type I cells is the presence of electron opaque dense cored vesicles (EDCV) in their cytoplasm; they were first described by Lever and Boyd in 1957. The distribution and number of the vesicles varies greatly from cell to cell and from area to area within the cell; they are spherical in shape, each consisting of a core of variable electron density surrounded by a lighter clear zone,

encased by a single trilaminar membrane (Al-Lami, 1964; Al-Lami & Murray, 1968a, 1968b). These vesicles are 200 - 2000 Å in diameter. The electron dense core is usually more prominent in glutaraldehyde-fixed tissue post-fixed in OsO₄ than in material fixed without glutaraldehyde (Garner & Duncan, 1958; Lever et al., 1959; Duncan & Yates, 1967; McDonald & Mitchell, 1975a).

Several authors have investigated whether the Type I cells form a single population based on the cytoplasmic electron density and/or the size and density of their constituent electron dense cored vesicles. In early electron microscopical studies Type I cells were classified as "light" (L), which contained a few EDVC and "dark" (D), which contained many EDVC (e.g. Lever & Boyd, 1957) However as pointed out by Biscoe (1971), most of the earlier attempts to subdivide the Type I cells were fruitless because of poor fixation of the material. However, this sub-division was also noted in the mouse and dog by Hoglund (1967), in the monkey by Kraus and Martinek (1967) and in man by Grimley and Glenner (1968). Based on light and electron microscopic studies, Kobayashi (1968) sub-divided the Type I cells of the dog carotid body into chromaffin cells, with extremely dense EDCV of irregular shape, and non-chromaffine cells with rounded particles EDCV of moderate electron opacity. These two types of Type I cells reported by Kobayashi (1968) cannot be explained as fixation artefact. Morita et al (1969) have divided the Type I cells of the cat into light and dark cells and subsequently sub-divided the dark cells into three types according to the number, size and density of their EDCV. Unfortunately, these authors did not indicate if the calculated

mean diameters were derived from one or several cells of each kind, they did not state the number of measured vesicles, nor did they describe the method of selecting cells. These inadequacies render the study valueless in quantitative terms. McDonald and Mitchell (1975a,b) and Hellstrom (1975a) introduced morphometry to the study of Type I cells and the question of whether they are an homogeneous population with regard to electron dense cored vesicles. McDonald and Mitchell (1975a,b) described two cell types and reported that the mean diameter of the large dense-cored vesicles of the cells called Type 'A' was nearly 30% larger than that of Type 'B' cells. Similar results were reported by Hellstrom (1975a), however McDonald and Mitchell's vesicles were some 50% larger than Hellstrom's!

In the rabbit carotid body Verna (1977) reported great variability in the size of EDCV, not only between cells but also within a given cell. He concluded that it is impossible to distinguish two kinds of Type I cell by comparing the mean diameter of their dense-cored vesicles. However, some cells seemed to contained more than one kind of EDCV; a more comprehensive analysis of this point is required. He also supposes that there is a morphologic evolution of Type I cells, which may be reflected by the size of the 'EDCV'. He explained the great variability in the EDCV diameter and the presence of unimodal bimodal or even three peaks in the diameter histogram **curve** by:

- 1) Type I cells, or at least some of them, contained different kinds of EDCV's, which differed in their mean diameter. However, the positions of the different peaks vary

from cell to cell and the relative proportions of the different populations of vesicles (if they really exist) also vary continuously from cell to cell. It is therefore impossible to distinguish clear-cut categories of Type I cells.

2) It is possible that Type I cells contain a single population of vesicles but that their distribution with regard to size is too large and not uniform in the cytoplasm. In this case the different peaks of the frequency curve would be artefacts and it would be necessary to measure a great number of vesicles in different areas of the cell before having a good representation of distribution.

B - Type II Cells

These cells are much smaller and fewer than Type I cells and are extensively branched with long cytoplasmic processes extending several times the cell body length and surrounding the Type I cells. The body of the cell is always located at the periphery of the lobules where the perilobular basement membrane separates the outer aspect from the interlobular connective tissue. The cells are flattened and finely granular with fewer organelles than the Type I cells. The nucleus, situated in the broadest segment of the cell is ovoid, reniform or triangular in shape and possesses a more uniform rim of chromatin material than Type I cells; organelles within the cytoplasm are located mostly in the perinuclear region (Al-Lami, 1964; Al-Lami & Murray,

1968a, 1968b; Biscoe & Stehbens, 1966). The Type II cells ensheath many unmyelinated nerve fibres of variable diameter and may provide a covering layer for some of the Type I cell nerve endings. Type II cells may function in a similar way to Schwann cells and glial cells in the central nervous system (Biscoe, 1971). The unmyelinated nerve fibres enclosed by Type II cell cytoplasm are of quite disparate size, ranging from some 1 μ to 0.1 μ m (Biscoe & Stehbens, 1966). The smallest fibres have been cited by Biscoe (1971) as possible afferent fibres. In this context it has been shown by Pallot (1974) that a number of these fibres terminate within the enclosing Type II cell without forming any specialised contacts. Due to the nature of such studies only four such fibres were positively identified; hence their physiological importance is uncertain.

1. 5. BLOOD SUPPLY

The carotid body receives its blood supply from either a branch of the external carotid artery or from one of the other major arteries arising at the termination of the common carotid artery. Even in a given species the precise topography of the supplying vessels is variable (see Seidl 1975).

The venous drainage of the organ joins either the external or the internal jugular veins, depending on the species involved (Chungcharoen et al., 1952; Daly et al., 1954).

Seidl(1975) described two different types of arterio-venous anastomoses in the cat carotid body:

(a) bridge anastomoses,

and (b)spiral anastomoses.

According to McDonald & Mitchell (1981) the venules account for only some 2% of vessels within the organ; they also described multiple parallel pathways between arterioles and venules in the carotid body and venous connections of the carotid body exhibit considerable redundancy so multiple pathways exist for the blood to leave the organ.

Some authors believe plasma skimming takes place in the carotid body; that is that the small capillaries are mainly perfused by plasma while the red blood cells are re-routed through shunt vessels (Acker & Lubber, 1976). This hypothesis was evoked to explain the observation by Acker and Lubber (1976) that

the PO_2 change in the carotid body after stopping perfusion was similar whether prior perfusion was with blood or saline. Verna (1981b) denied the presence of plasma skimming in the rabbit carotid body. More recent quantitative studies (Pallot & Verna, in preparation) have shown that the Vv% of red blood cells within the specific tissue is unaffected by the tension of O_2 in the inspired gas.

In proportion to its weight, the carotid body has the largest blood flow and oxygen consumption of any tissue in the body, nearly 2000 ml/100 gm C.B./minute (Daly et al., 1954; Keller & Lubbers, 1972). In comparison blood flow through the brain is approximately 60 ml/100 gm tissue/minute (Dumke & Schmidt, 1943), while in the heart, blood flow is approximately 64 to 151 ml/100 gm tissue/minute (Gregge, 1950). Other authors (Fay, 1970, Leitner & Liaubet, 1971) described lower values for carotid body blood flow but still high when compared to other organs. They described a high oxygen consumption by the carotid body - 7.5 times greater than that in the cerebral cortex. The oxygen uptake is about 9 ml/100 gm carotid body/minute whilst it is approximately one third of that value in the brain (Ketz & Schmidt, 1945).

1. 6. INNERVATION

The carotid body of mammals is principally innervated by the carotid (sinus) nerve (CSN) - a branch of the

glossopharyngeal nerve (de Castro, 1926). It also receives fibres from the superior cervical ganglion (see Biscoe, 1971). In birds, the nodose ganglion of the vagus nerve (CN X) is the main source of carotid body nerve fibres (Jones & Purves, 1958).

The carotid nerve contains both myelinated and unmyelinated fibres which enter the carotid body (Gallego & Belmonte, 1983; Eyzaguirre & Uchizono, 1961). Myelinated fibres lose their myelin sheaths and ultimately become enveloped by the processes of the Type II cells. The Type II cells of the carotid body do not receive any specific innervation but there is some anatomical evidence supporting the idea that fine nerve fibres may end enclosed in Type II cell cytoplasm without first making specialised contacts (de Kock & Dunn, 1966; Pallot, 1976).

Generally a single nerve fibre may form a number of endings on a single Type I cell as well as several Type I cells of a group (Biscoe & Pallot, 1972; Kondo, 1976b; Nishi & Stensaas, 1974).

Endings on Type I cells may take several forms. Generally the synapse contains varying number of small (clear-cored) vesicles (350 - 700 Å in diameter), multivesicular bodies, variable numbers of mitochondria and glycogen granules and a few dense cored vesicles (450 - 750 Å in diameter) (Ishii & Oosaki, 1966; Biscoe & Stehbens, 1967; Kobayashi & Uehara, 1970; McDonald & Mitchell, 1975a,b; Morgan et al., 1975; Kondo, 1976a,b).

Regions of specialised membrane contact between nerve

endings and Type I cells may be found (see Fig. 1.1). The specialised region is characterised by an increase in the membrane thickness on both sides of the contact, accompanied by increase inter-membrane distance (300\AA apart); a fine line of dense material may be seen between the two membranes. This membrane specialisation may be one of four types:

a) the first type is characterised by asymmetrical, uniform membrane thickenings with accumulations of clear vesicles in the region of the membrane thickening on the nerve ending side. The nerve membrane bears small, dense serrations (pre-synaptic dense projections) (Gray, 1962, 1963). This type of ending is thought to be efferent (Biscoe & Stehbens, 1966; Hoglund, 1967; Grimley & Glenner, 1968; Ishii & Oosak, 1969; Kobayashi, 1968, 1969, 1971b; Kondo, 1971; Morgan et al., 1975; McDonald & Mitchell, 1975).

b) the second type is characterised by asymmetrical membrane thickenings, those of the nerve endings being uniformly thick and those of the Type I cells containing pre-synaptic dense projections. Whilst vesicles are seen on ^{the} nerve endings side, accumulations of dense cored and clear vesicles (500\AA in diameter) are seen on Type I cell side. This type of ending is thought to be afferent (Al-Lami & Murray, 1968b; Ishii & Oosaki, 1969; Kobayashi, 1969; Kondo, 1971; Morgan et al. 1975).

c) a third type of specialised contact is occasionally found in both cat and rat carotid bodies (McDonald & Mitchell, 1975a,b) the so-called reciprocal synapse (see Reese &

FIGURE 1.1 DIAGRAM SHOWING THREE TYPES OF TYPE I - CELL -
NERVE ENDING JUNCTIONAL SPECIALISATION

- a) Efferent (ending presynaptic to Type I cell)
- b) Afferent (ending postsynaptic to Type I cell)
- c) Reciprocal (ending pre and postsynaptic to
Type I cell)

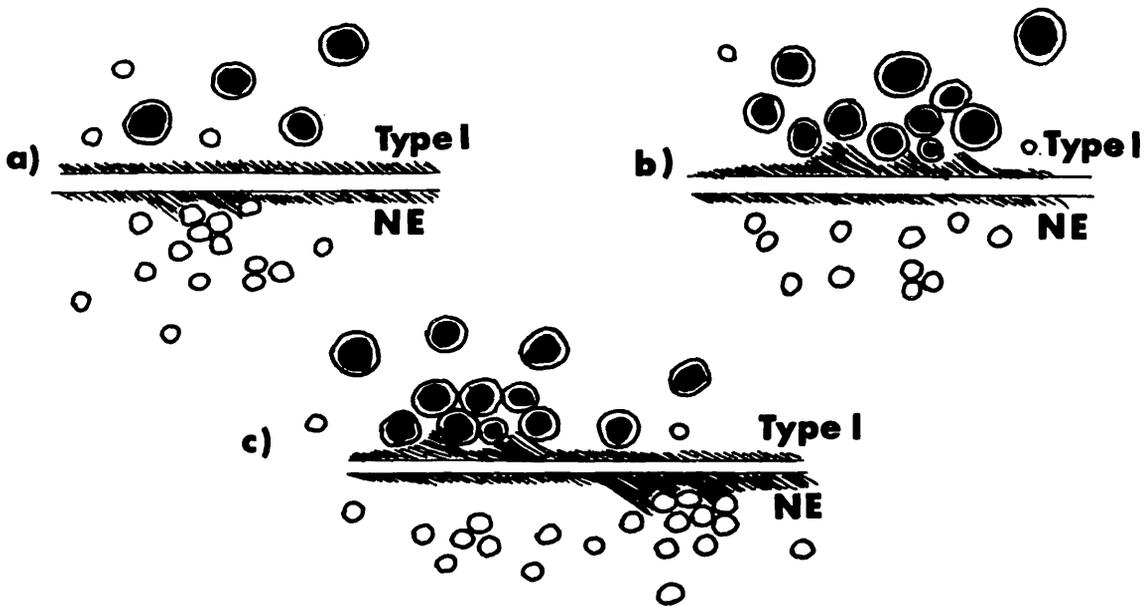


FIGURE 1.1

Shepherd, 1972). In this case pre-synaptic dense projections are found on both the nerve and Type I cell membranes and it is suggested that at one region the ending is pre-synaptic to, and at the other post-synaptic to, the Type I cell.

d) endings where the membrane thickenings are symmetrical.

Quantitative studies of the types of nerve endings occurring on Type I cells of the cat and rat carotid body have been performed (McDonald & Mitchell, 1975a,b, 1981; Pallot & Blakeman, 1982 and see below).

Despite these differing ultrastructural features many authors assumed that all Type I cell endings were sensory; however the presence of synaptic vesicles in the nerve endings on Type I cells led to suggestions that these nerve were structurally more like efferent rather than afferent endings (Biscoe & Stehbens, 1966; Abraham, 1968).

Following on essentially from their own physiological observations Biscoe, Lall and Sampson (1970) repeated the intracranial nerve section experiment. Briefly, they claimed that some 60% of nerve endings on Type I cells degenerated with a slow time course of several months. During this period normal chemoreceptor activity could be recorded. The loss of nerve endings, allied to an essentially normal chemoreceptor discharge led these authors to suggest that the Type I cell nerve endings were efferent.

A number of authors have now performed this intracranial section experiment (Hess & Zapata, 1972; Nishi & Stensaas, 1974; McDonald & Mitchell, 1975a,b). All claim to confirm the result of de Castro (1928). Nishi and Stensaas (1974) and McDonald and Mitchell (1975a,b) used only short recovery times after surgery despite the fact that Biscoe et al. (1970) specifically remarked on the unusually long time course of degeneration. Hess and Zapata (1972) on the other hand used longer recovery times; one of their illustrations, taken some 60 days after operation, shows a markedly abnormal structure which is claimed as a normal nerve ending. Whether this structure indeed represented a degenerating nerve ending or was merely the result of inadequate fixation is not clear.

With hindsight it must be stated that the study of Biscoe et al. (1970) is unsatisfactory in a number of respects. The question of normality of discharge is obviously highly subjective. Thus, it is claimed that damage to the sensory ganglion explains the degeneration (e.g. McDonald & Mitchell, 1975) and that a 60% loss of endings does not produce detectable changes in chemoreceptor activity by the methods currently available. Where the study of Biscoe et al. (1970) could have been much improved is by a count of sensory ganglion cells and nerve fibres in the sinus nerve. Neither was attempted.

The problems associated with degeneration experiments were apparently circumvented by autoradiographic studies. Tritiated proline (Fidone et al., 1975, 1977) and tritiated leucine (Smith & Mills, 1976) were injected into the petrosal ganglion of the

cat in the jugular foramen. Subsequent electron microscopic autoradiography showed that labelled substances were located in the region of both the myelinated and unmyelinated fibres entering the carotid body and also close to the endings on Type I cells. The results suggest that some of the endings on Type I cells arise from the petrosal ganglion. Tradition would have us believe that this ganglion is formed exclusively by sensory unipolar neurons.

Despite the claims made for autoradiographic studies it must be emphasised that by their very nature they are not capable of producing quantitative data with regard to numbers of endings labelled. Furthermore, as pointed out by Williams et al.(1977) the mere examination of autoradiographs is an inadequate test of whether a structure is labelled. Various techniques exist for the detailed analysis of autoradiographs - none of these was applied in the studies referred to above and, hence, the confidence limits of the study are not established.

Horseradish peroxidase transport techniques have also been used in studying this problem with variable results. Some workers claim that only areas containing established sensory neurons are stained (Kalia & Davis, 1978) whilst others suggest an efferent component in the sinus nerve (de Groat,1979)

In attempts to examine this question further Biscoe and Pallot (1975, 1983) studied the carotid bodies from the mutant Wobbler mouse. This animal has a genetic defect which results in the spontaneous degeneration of its motor neurons. Quantitative

ultrastructural studies demonstrated that a very large percentage of Type I cell nerve endings were lacking in this animal (60 - 90%). Physiological studies, conducted in the same animals, showed that the animals:

- a) increased their minute volume in response to hypoxic stimulus;
- b) responded to one or two breaths of 100% O₂ with a brief reduction in minute volume.

Both of these effects were abolished by sinus denervation. These observations are, unfortunately, again equivocal. Thus, at first sight they suggest that many of the Type I cell nerve endings are efferent, rather than afferent. However, there still remains the question of redundancy and the possibility that whilst the endings are destroyed the parent axons remain intact and act as chemoreceptors.

What was needed was a more physiological type of experimental approach. Such was attempted by Morgan, Pallot and Willshaw (1976, 1981) and Pallot, Morgan and Willshaw (1981). It was shown by McDonald & Mitchell (1975a,b) that in the rat the synaptic vesicle content of Type I cell nerve endings was effected by the inspired gas mixture so that 100% O₂ increased vesicle density whilst 10% O₂ reduced it over room air control values. Morgan et al. ventilated adult cats with either 10% O₂ in N₂ or 100% O₂. One carotid body was innervated by an intact sinus nerve whilst the contralateral nerve was sectioned immediately prior to ventilation with the test gas. After 100% O₂ there were

always fewer synaptic vesicles in the sectioned nerve Type I cell endings than the contralateral intact side. After 10% O₂ the situation was reversed, viz: the sectioned nerve side endings now contained more vesicles than those on the contralateral intact side. Furthermore, the concentrations of vesicles in the cut side nerve endings are similar after 10% and 100% O₂. These results demonstrate that some descending influence relayed via the sinus nerve affects vesicle content of the nerve endings; in other words, that the carotid sinus nerve relays efferent fibres to the cut carotid body. In view of the quantitative morphological studies (Pallot & Blakeman, 1982) it seems likely that these efferent fibres in the cat carotid body terminate on Type I cells.

Kondo (1976a) examined the development of rat carotid body ultrastructurally. He described two types of synapses - efferent and afferent. The afferent synapses were more numerous than the efferent ones and, as development proceeds, the afferent synapses increase in number whilst the efferent increase was very little until 50 days after birth at which time the afferent synapses are 22 times more numerous than the efferent. Kondo suspected this was due to an increase in the number of synapses per nerve fibres, rather than an increase in the number of nerve fibres.

Recent studies by McAllen and Willshaw (1979) have demonstrated that acute intracranial section of the CN IX nerve does not abolish the efferent activity recorded from the central cut end of the sinus nerve. This study suggests, therefore, that the cell bodies of the efferent neurons are located peripherally

rather than in the brain stem. If true, this observation is of considerable importance.

Can we correlate all of the above data with the ultrastructural features referred to above?

McDonald and Mitchell (1975a, b) performed an extensive quantitative study of the rat carotid body. These authors described endings that were:

a) pre-synaptic to Type I cells;

b) post-synaptic to Type I cells;

c) endings where the pre-synaptic dense projections were found in both the neural and cellular elements.

McDonald and Mitchell (1975a, b) also demonstrated that section of the sinus nerve resulted in the loss of 95% of Type I cell nerve endings and also that section of the pre-ganglionic trunk, as well as removal of the superior cervical ganglion, resulted in degeneration of a small number of nerve endings on Type I cells, thus accounting for the remaining terminals observed after peripheral section of CN IX. They further postulated that both pre-ganglionic sympathetic efferent terminals and glossopharyngeal afferent nerve terminals were present on Type I cells in the carotid body. The fine structure of these terminals was examined with quantitative methods and it was concluded that sympathetic afferent and CN IX Type I cell sensory nerve terminals could be differentiated on the basis of differences in

synaptic vesicle size and density.

The hypothesis from these experiments was that most, if not all, of the endings in the sinus nerve were sensory (because they survived 10 days after intracranial section of CN IX); the pre-synaptic (to Type I cells) were present because the second element of the reciprocal synapse was not visible in that particular plane of section.

These results, with regard to percentage of the different junctional complexes in the carotid body have been confirmed by Pallot & Blakeman(1982); in addition, these authors have demonstrated that ventilation with 100% and 10% O₂ does not produce different effects on the vesicle content of the nerve endings depending on the the state of the sinus nerve and, hence, the rat apparently lacks any substantial efferent innervation via the sinus nerve.

Quantitative studies of cat Type I cell nerve endings (Pallot & Blakeman, 1982,) have yielded quite different results. In this studies of several thousand nerve endings some 93% lacked any membrane specialisation, 4.9% were pre-synaptic to Type I cells, 1.5% were post-synaptic to Type I cells and some 0.6% formed reciprocal synapses. From this data, showing a predominance of apparently efferent endings, coupled with the effects of 100% and 10% O₂ on the vesicle density of nerve endings, the authors concluded that it seems likely that a number of Type I cell nerve endings, perhaps some 50%, are truly efferent; also they found no evidence for strict division of the

endings to small bouton-like terminals and large calyceal endings as has been claimed by some authors (Kondo, 1976a,b; Nishi & Stensaas, 1974).

Nerve to nerve endings have occasionally been reported (Verna, 1975; Morgan et al. 1975). These observations are of considerable interest because of the controversy over the efferent pathway (discussed below). The major problem here lies in the identification of nerve ending profiles. Thus it is relatively simple to identify a nerve ending on a larger Type I cell profile; regrettably, however, the Type I cell often possesses thin cytoplasmic processes containing clear vesicles, glycogen granules and mitochondria. If one of these processes is contacted by a nerve ending it is impossible to elucidate the ultrastructural picture without serial reconstruction studies. These have not been performed.

Although the presence of autonomic ganglion cells in the carotid body was well documented by early light microscopic studies, McDonald and Mitchell (1975a,b) and Kondo (1976b) were the first to describe the ultrastructure of these cells in detail. McDonald and Mitchell (1975) considered most ganglion cells to be parasympathetic in nature and to provide post-ganglionic axons which innervated the vascular smooth muscle. They also noted a few sympathetic ganglion cells which were innervated by pre-ganglionic axons from the superior cervical ganglion. Again, these cells gave a post-ganglionic vasoconstrictor innervation to the vasculature. In their degeneration studies McDonald and Mitchell claim also to have

found sympathetic pre-ganglionic endings which were pre-synaptic to the Type I cells and apparently did not innervate blood vessels. These endings were considered to be the only truly efferent innervation to rat Type I cells. Some authors (Kondo, 1971; Verna, 1975) have shown that post-ganglionic sympathetic terminals may be seen also close to Type I and Type II cells. Verna (1979) demonstrated that varieties of post-ganglionic sympathetic nerve fibres are present not only around blood vessels but also around Type I/Type II clusters and may even be in contact with Type II cells. It may be seen that no clear picture with regard to Type I cell sympathetic innervation has appeared. It is worth mentioning, however, that O'Regan (1977) has reported physiological results which strongly suggest a Type I cell sympathetic innervation.

1. 7. FACTORS AFFECTING THE CAROTID BODY STRUCTURE

1. 7. 1. HYPOXIA AND ANOXIA

Hypoxia, both acute and chronic, cause changes in the content and structure of the carotid body. Only the effects of acute exposure will be discussed here.

Blumcke et al. (1967) reported that, after ten minutes of 2.5% O₂, the electron dense cored vesicles were decreased in number by 'reverse pinocytosis'. They claimed that the vesicles fused with the plasma membrane, ruptured and released electron dense material into the intercellular space. Along with these

changes the mitochondria were swollen, nuclear chromatin disintegrated and the nuclear pores increased in number. Fluorescence in these cells disappears after twenty minutes of hypoxia. To the present author these changes were probably caused by cell death; indeed it is doubtful if many of the animals survived such insults. Hoffmann and Birrell (1958) and Mitchell and McDonald (1975) also noted a decrease in the number of vesicles in the Type I cells following hypoxia using more physiological stimuli. Chen et al. (1969) did not see a decrease in electron dense cored vesicles in anoxic hamsters although the mitochondria were swollen, whilst Al-Lami and Murray (1968a) claimed that there is a relative increase in the number of such vesicles in cats subjected to severe anoxia together with swollen mitochondria.

On the other hand, Moller et al. (1974) claimed that the number of dense cored vesicles showed a marked increase in rabbits exposed to hypoxic conditions, suggesting an increase in the production of catecholamines during hypoxia.

From this brief resume it is apparent that there is no coherent picture of the effects of acute hypoxia on carotid body structure. It seems important therefore that this situation be rectified and that the experiments be performed using sensible, physiological stimuli.

1. 7. 2. RESERPINE

Studies on the effects of reserpine on the carotid body

provided both interesting and controversial results.

Amine depleting drugs like reserpine may operate by one of five different mechanisms. They may:

1. inhibit the synthesis of catecholamines;
2. inhibit their storage mechanism;
3. cause displacement of their storage sites;
4. cause cell damage leading to cell degeneration;
5. result in stimulation and subsequent physiological release of catecholamines (Carlsson, 1975).

Reserpine acts by blocking the ATP-dependent uptake mechanism by which monoamines are incorporated into a complex ATP and a specific protein and sequestered in protective vesicles. Catecholamines within the cytoplasm are then subjected to the cell monoamine oxidase which de-aminates and metabolises these substances resulting in depletion of catecholamines within the cell and its vesicles (Carlsson, 1975; Hess, 1977a); it is known to deplete the catecholamine content of the cells containing adrenaline and noradrenaline and also enterochromaffin cells (Lillie, 1965).

The normal catecholamine fluorescence of the carotid body disappears after reserpine treatment yet granular profiles still remain within the Type I cells unlike the situation which exists

in the adrenal medulla where the granules are entirely depleted (Duncan & Yates, 1967; Chen et al., 1969; Hess, 1977; Mollmann et al., 1972; Chiocchio et al., 1971).

Hess (1976, 1977b) has shown that calcium prevents reserpine depleting catecholamines from the Type I cells and is effective against doses not higher than 5 mgm/kgm. Calcium might counteract the effects of reserpine by occupying attachment sites of vesicular membrane and thereby preventing it from reaching its site of action. In his morphometric study on the effect of reserpine on the carotid body, Hess (1976; 1977b) claimed that the numerical density of the electron dense cored vesicles remains unchanged but their volume density is reduced. The persistence of dense granules in Type I cells after reserpine treatment could be due to several factors. The effect of reserpine on the carotid body may result from the binding between the catecholamines and other components of the storage vesicles being more resistant to its action. This was supported by Coupland and Hopwood (1966) who showed that there is intense reaction between gluteraldehyde and noradrenaline resulting in the formation of a highly insoluble electron dense compound. A second possibility is that the catecholamines and other components leave the vesicles or are made non-reactive to the effect of fixatives by some other mechanism. A third possibility is that the vesicles may contain a binding protein which, with the catecholamines, makes a complex that remains within the vesicles when the catecholamines are depleted. A fourth possibility is the existence of a polypeptide hormone produced and stored by the carotid body which remains unaffected by

reserpine treatment.

1. 7. 3. STEROIDS

Administration of glucocorticoids, dexamethazone (Hellstrom & Koslow, 1976) methylprednisolone and hydrocortisone (Korkala et al., 1973), caused an increase in the fluorescent intensity of carotid Type I cells (Korkala et al., 1973) as well as an increase in the volume density of the dense cored vesicles of the Type I cells (Hellstrom & Koslow, 1976). Glucocorticoids most likely cause an increase in the storage of catecholamines possibly by stimulating synthetic enzymes.

1. 8. CATECHOLAMINES AND THE CAROTID BODY

Catecholamine-containing cells synthesise their endogenous amine content of dopamine (3,4-dihydroxy-B-phenylethylamine; 3-hydroxytyramine) (DA), L-noradrenaline (atrenol; norepinephrine) (NA) and L-adrenaline (epinephrine) (AD) from the plasma amino acid L-tyrosine by a pathway first suggested by Blaschko in 1939, viz: L-tyrosine \longrightarrow L-dopa \longrightarrow Dopamine \longrightarrow L-noradrenaline \longrightarrow L-adrenaline (Iversen, 1970).

The fact that the carotid bodies of all species studied to date contain catecholamines has been reported above. Firstly, there was the controversy as to the chromaffinity, or otherwise, of Type I cells (e.g. Stilling, 1898; Kose, 1907) and, more recently, the demonstration of formaldehyde -induced fluorescence (e.g. Palkama, 1965). This cytochemical work has been followed up in the last fifteen years by numerous chemical determinations of carotid body catecholamine levels.

Noradrenaline and dopamine were found to be the main catecholamines in several mammalian species (Chiocchio et al., 1966; Dearnaley et al., 1968; Hellstrom & Koslow, 1975; Hellstrom et al., 1976, 1977a,b; Mills et al., 1978) whilst, in the carotid labyrinth of Rana temporaria adrenaline is the predominant catecholamine, with traces of dopamine and noradrenaline (Bannister et al., 1967).

In 1964 Niemi and Ojala obtained cytochemical evidence for the presence of catecholamines in the human carotid body.

Chiocchio et al. (1966) found adrenaline, noradrenaline and dopamine in the carotid body of the cat and they suggested that dopamine, which was present in a concentration of 0.1 to 0.2 ng/carotid body, might be stored in mast cells in the tissue and that it may be the precursor of other catecholamines. Using a trihydroxyindole procedure to measure the rabbit carotid body catecholamine levels, Dearnaley et al. (1968) estimated the dopamine level to be 20 to 40 ug/g and the noradrenaline level to be about 1.5 ug/g of carotid body tissue. Furthermore, they found that there were no fluorescent mast cells in the organ and, due to the high levels of dopamine found, suggested that dopamine is of more significance in the carotid body other than as a metabolic intermediary.

The literature on the catecholamine content of carotid bodies is vast, confusing and embittered with claim and counter-claim. Table 1.1 illustrates the results of studies of catecholamine levels in several species up to 1982.

The data from these reports are difficult to interpret because the differences seen within the same species, or within different reports from the same laboratory, could be due to:

1. differences in the sensitivity of the method employed;
2. the physiological stimulus in normal conditions;
3. statistical errors in data and interpretation;
4. other factors.

ANIMAL	REPORT	NA	DA
CAT	Chiocchio et al (1966)	479	875(d)
	Zapata et al (1969)	579	1331(d)
	Mills et al (1978)	957	221(e)
	Mir et al (1982)	820	162(b)
		860	168(c)
RAT	Hellstrom & Koslow (1975)	8.5	14.5(a)
	Hellstrom et al (1976)	3.6	15.0(a)
	Hellstrom (1977)	3.6	14.5(a)
	Hanbauer & Hellstrom (1978)	2.5	8.5(a)
	Chiocchio et al (1981)	4.5	5.1(b)
	Mir et al (1982)	14.6	8.1(c)
	Hansen et al (1981)	9.7	14.2(c)
RABBIT	Dearnaley et al (1968)	-	137(e)
	Sorensen (1967)	72	294
	Mir et al (1982)	84	216(c)
GUINEA-PIG	Mir et al (1982)	218	46(c)
FERRET	Mir et al (1982)	15	105(c)

Table 1.1

Catecholamines in the cat, rat, rabbit, guinea-pig and ferret carotid body.

Values expressed as p mol/carotid body.

- (a) gas chromatography and mass spectrometry;
- (b) radioenzymatic assay;
- (c) HPLC and electrochemical detection;
- (d) fluorimetric analysis;
- (e) fluorimetric analysis and ion exchange chromatography.

Some of the methods employed (such as the trihydroxyindole technique used by Dearnaley (1968) and Mills & Slotkin (1975)) measure the total noradrenaline and adrenaline; others, such as that used by Chiocchio et al. (1971), give values for dopamine and L-dopa together. The gas chromatographic and mass spectrometric assay method used by Hellstrom et al. (1975, 1976, 1977a,b) might affect the dopamine value estimation since this method includes an acid lyophilization step which may cause release of free dopamine from the conjugated form of this amine present in plasma contained within the carotid body (Mir et al., 1982). The fluorimetric procedures used in earlier studies (Chiocchio et al., 1966; Zapata et al., 1969) are not only less sensitive but also lack specificity (Mills et al., 1978).

The difference in the noradrenaline content of the rat carotid body as reported by Hellstrom et al. (see Table 1.1) may not be explained by variations in the endogenous noradrenaline content found within different groups of animals (Mills et al., 1978) but may reflect rather the action of a physiological mechanism operating to deplete noradrenaline while the animal breathed room air.

Statistical errors in reports and in data interpretation were other factors in this morass^{of} reports. For example, the catecholamine (adrenaline, noradrenaline and dopamine) contents of the cat carotid body reported by Chiocchio et al. (1966) were approximately 30 times lower than those reported in their previous study (Chiocchio et al, 1960) and are probably below the

sensitivity of the assay method. Also Chiocchio et al. (1971) reported dopamine values in two pooled samples of cat carotid bodies to be 122 - 131 ng/carotid body but it is stated that these values are inclusive of any L-dopa present. When separation on Dowex ion exchange resin was used the value for L-dopa was found to be 86 - 150 ng/carotid body and thus it would seem that subtraction of this value from that given for dopamine plus L-dopa should yield a very low figure for dopamine alone (0 - 50 ng/carotid body). However, in the text, the value of dopamine alone is given as 260 ng/carotid body whilstⁱⁿ the Abstract (Chiocchio et al. 1971) dopamine as 122 - 131 ng/carotid body.^{given}

Other factors which may affect the catecholamine content of carotid bodies include methods of sacrificing the animals, the animal strains, number of estimations, and dissection procedures.

There is also considerable confusion and controversy in the literature concerning the relative concentrations of noradrenaline and dopamine in the rat carotid body. For example, dopamine was reported to be the predominant amine in the carotid body of the cat (Chiocchio et al., 1966; Zapata, 1969) and rat (Hellstrom & Koslow, 1975; Hellstrom et al., 1976; Hellstrom, 1977a,b; Hanbauer & Hellstrom, 1978) whereas Mills et al (1978) and Mir et al. (1982) have reported that the cat carotid body contains substantially higher concentrations of noradrenaline than dopamine. Similar findings were reported in the rat carotid body by other authors (Al-Neamy et al., 1980; Mir et al., 1982).

The results of the work by Chiocchio et al. (1966) show

that, despite the dopamine level being higher than that of noradrenaline (in the cat carotid body) the difference calculated from the mean and standard error of the raw data is not significant. Hellstrom and Koslow (1975) also reported in the rat carotid body a higher level of dopamine as compared with noradrenaline but the standard error estimated from the published bar graph suggests the difference between the noradrenaline and dopamine content to be not significant.

In contrast to the cat and guinea pig carotid bodies, those of the ferret and rabbit appear to contain predominantly dopamine.

The situation is obviously confused, and requires a systematic study.

1. 9. FACTORS AFFECTING THE CAROTID BODY CATECHOLAMINE LEVEL

1. 9. 1. HYPOXIA

In the cat carotid body systemic hypoxia (10% O₂ for 60 minutes) was reported by Mills and Slotkin (1975) to cause a significant reduction in noradrenaline and adrenaline content (measured together). They also reported that the reduction was greater when the inspired oxygen was less and that the depletion of these catecholamines was markedly attenuated, but not prevented completely, when the carotid sinus nerve was cut acutely just prior to the hypoxic tests. The dopamine level was not measured separately in their study. In the rat carotid body

Hellstrom and Koslow (1975), Hellstrom et al. (1976), Hellstrom (1977a) and Hanbauer and Hellstrom (1978) all reported that systemic hypoxia (5% O₂ for 30 minutes) had no effect on noradrenaline levels, while the dopamine content was significantly reduced. They also claim that the reduction was dependent upon the severity and duration of the stimulus and that sectioning of the sinus nerve and sympathetic denervation had no effect on the amount of the dopamine reduction. Hence, it seems that systemic hypoxia (short-term) causes a selective reduction of the catecholamine level which may be dependent on the sinus nerve innervation in the cat but not in the rat.

In marked contrast, chronic hypoxia is reported to cause an increase in both noradrenaline and dopamine (Chiocchio et al., 1981; Pallot & Barer, 1982).

1. 9. 2. HYPEROXIA

Mills and Slotkin (1978) reported an increase in noradrenaline and adrenaline content of the cat carotid body following ventilation with 40% O₂ for 90 minutes while, in rats, Hanbauer and Hellstrom (1978) reported no effect of hyperoxia (100% O₂) on either noradrenaline or dopamine content.

Despite the above findings no systematic study on the effects of hypoxia, hyperoxia and hypercapnia on carotid body catecholamine content has been carried out.

1. 9. 3. SYMPATHECTOMY

In the rat, Hellstrom and Koslow (1975) and Hellstrom (1977b) found that neither sympathetic denervation nor chemical sympathectomy (6-OHDA) had any effect on the carotid body catecholamine content. In later reports, however, Hanbauer and Hellstrom (1978) showed a 50% reduction in noradrenaline content following ganglionectomy, whilst dopamine content was not affected. Chiocchio et al. (1981) also reported a 75% loss of noradrenaline in the rat carotid body following ganglionectomy. Zuazo & Zapata (1978) reported that sympathectomy had no effect on cat carotid body catecholamines, a fact confirmed by Mir et al. (1982).

1. 10. _____ MECHANISM OF CHEMORECEPTION AND EVIDENCE CONCERNING THE IDENTITY OF SENSORS

Despite the many studies of the carotid body there is still no all-embracing hypothesis as to how it functions and, indeed, there is still controversy concerning which structure within this organ acts as the sensory transducer.

One of the central issues concerns the identity of the chemoreceptor; at different times each of the specific structural entities (Type I cells; nerve endings and nerve fibres; Type II cells) have been proposed as the receptors.

1. 10. 1. TYPE I CELLS AS CHEMORECEPTORS

Historically, and on the basis of their close association with nerve ENDINGS, the Type I cells have been considered as receptor cells which 'taste the blood' (de Castro, 1926; 1928). In this theory the Type I cells are sensory cells. Eyzaguirre et al. (1965b;1968b) suggested that the Type I cell releases acetylcholine in response to hypoxia which, in turn, stimulates afferent nerves. The evidence for this suggestion came from a Loewi-type experiment. Subsequent studies (e.g. Fidone et al. 1977) have, indeed, demonstrated the presence of acetylcholine within the carotid body. However, the difficulty here is that Douglas (1952) and Sampson (1971, 1975) could block the effects of exogenous acetylcholine pharmacologically but could not prevent the chemoreceptive response to hypoxia. So, is the Type I cell necessary for chemoreception? Most of the evidence currently cited in support of the view that the Type I cell is a sensor candidate comes from three types of experiments:

1. Re - innervation of the carotid body with a foreign nerve

Three groups of workers have performed this type of re-innervation experiment: de Castro et al. (1940; 1951) used the vagus nerve; Zapata et al. (1969) used the superior laryngeal nerve and Diger et al. (1983) used the lingual nerve. All of them demonstrated chemosensitivity in the re-innervated organ. The criticism that the first two groups had used nerves known to contain some fibres that responded to changes in inspired gas tensions was overcome by Fidone et al. (1983) when they used the lingual nerve.

These experiments undoubtedly establish that single fibres which have been grown into the carotid body from a foreign nerve can exhibit an essentially normal chemoreceptor activity when compared with single sinus nerve fibres. In the absence of quantitative structural data the assertion that there appears to be a relationship between the amount of chemoreceptor activity and the number of contacts between Type I cells and nervous elements is purely subjective. The cited authors interpret their findings as indicating that the Type I cells are essential for chemoreception.

2. Re - innervation of the carotid body with its own sinus nerve

The results of these experiments were somewhat equivocal. Zapata et al. (1976; 1977) crushed the sinus nerve at various distances from the carotid body and reported that chemoreceptor activity returned in parallel with the appearance of new neural contacts with Type I cells. However, Kienecker & Knoche (1977) and Bingmann et al. (1977) have sectioned the sinus nerve and anastomosed the cut ends. They reported different results from those of Zapata. From their data it seems that activity returns prior to there being contact between nerve and Type I cells. Belmonte et al. (1981) reported his failure to record chemoreceptor activity six months after re-innervation of the superior cervical ganglion with the sinus nerve.

3. Experiments in which various manoeuvres have been used to destroy or damage the Type I cells

Such experiments have been attempted by Verna et al. (1975) and Leitner et al. (1981), using a freezing technique, and Monti-Bloch (1981) using an ischaemic preparation. After Type I cell destruction no chemoreceptive activity was recorded in the carotid sinus nerve.

Superficially, all these data support the view that Type I cells are essential for chemoreception; however, other interpretations are possible. The re-innervation experiments are, to some degree, uncontrolled for when the nerve enters the carotid body it is not only coming into contact with the Type I cells but also with all the other cellular elements. It is claimed that endings must make contact with Type I cells (Zapata et al., 1976; 1977) but no quantitative data is given to support this contention. Regarding the attempt to remove or damage the Type I cells by freezing, the fact that some activity remains if a proportion of the cells survive is difficult to interpret for - without extensive quantitative structural studies - there is no way of establishing whether other conditions are changed within the frozen organ. Gual & Stensaas(1983) have shown that disrupting the Type I cells by barium ions does not lead to a loss of chemosensitivity. In Wobbler mutant mice Pallot and Biscoe (1977) reported that the Type I cells were almost totally devoid of nerve endings but possessed a normal chemoreflex. In a later publication Biscoe and Pallot (1983) illustrated that it was possible to record chemoreceptor activity from the sinus nerve of these animals. Such data is, of course, very difficult to interpret but it would seem unlikely that Type I cells are essential for chemoreceptor activity when it is possible to

record such activity in animals where the Type I cells have virtually no innervation.

The common factor suggested by Pallot (1983) is that chemoreceptor activity can only be recorded when the nervous elements are placed in conditions similar to that of the carotid body and, although the above experiment has not established which feature of the organ provides this, a likely candidate is the highly vascularised environment.

1. 10. 2. NERVES AS CHEMORECEPTORS

The role of sensory endings in the carotid body as primary chemosensors has been suggested by a number of authors. Biscoe (1971) proposed 'that the receptor is a nerve fibre terminal surrounded by Type II cells' and he concluded in his 'small free nerve fibre' hypothesis that hypoxia could sufficiently depolarize small fibres as to produce action potentials with a response speed comparable to that observed in recordings from chemoreceptor fibres. The experimental data regarding this theory has been discussed previously.

The most favourable data for the nerve fibres as the primary chemoreceptors comes from neuroma experiments. The first of such experiments were performed by Mitchell et al. (1972) and, subsequently, similar experiments have been performed by Bingmann et al., (1978; 1981a,b; 1983), Kienecker et al. (1977; 1981; 1983), Tan et al. (1981) and Smith and Mills (1981).

The brief report by Mitchell et al. (1972) indicated that neuromas of the carotid sinus nerve exhibited chemoreceptor activity in the absence of Type I cells as assessed by fluorescence microscopy. The studies of Kienecker's group were far more systematic. Their physiological and structural studies of the regeneration products six months after implanting the sinus nerve into the adventitia of the external carotid artery showed two types of product could be distinguished structurally. One was nerve fibres which had grown into a remarkably vascular connective tissue (Kienecker & Knoche, 1981b) and exhibited chemoreceptor activity (Bingmann et al., 1981; 1983); the second showed unmyelinated nerve fibres intermingled with connective tissue in a manner not unlike that seen in the carotid sinus; physiologically, these products exhibited mechano-receptor properties (Bingmann et al., 1981a,b; 1983). Similar results were obtained by Tan et al. (1981). Smith and Mills (1981) permitted the sinus nerve to regenerate into connective tissue after removal of the carotid body. Under these conditions chemoreceptor activity did not appear in the nerve even six months after operation.

In Bingmann et al. (1981a,b) there is a possibility that the re-innervation of the carotid body is responsible for chemoreceptor activity but this is unlikely as Kienecker has shown that the rabbit carotid body degenerates after denervation. Pallot (personal communication) looked for carotid body tissue in the material studied by Tan et al. (1981) but without success. Later, Bingmann et al. (1983) produced an identical result from an experiment where the carotid body was removed. There is still

a possibility that isolated collections of glomus tissue become innervated by regenerating fibres and produce the recorded activity reported by Pallot (1983). The failure of the Mills and Smith (1981) neuromas to exhibit chemoreceptor activity is interesting; it must be said that the neuromas lacked the extensive vasculature reported by Kienecker's group.

Pallot (1983) suggests that nerve fibres and/or endings are able to generate chemoreceptor activity if they are in a highly vascular environment and the fact that the responses recorded by Bingmann et al. (1981a,b; 1983) are rather sluggish suggests that some other factor - presumably supplied by some element of the carotid body - is necessary for a normal response. In support of this are the results of Dinger et al. (1982a, 1983) who have shown that the lingual nerve, on reaching the region of the Type I cells, supports chemoreceptor activity. The normal chemoreceptor nerve, however, does not show chemoreceptor activity when the taste buds are innervated.

1. 10. 3. THE TYPE II CELLS AS CHEMORECEPTORS

The Type II cell has also been postulated as a chemosensor. Whilst all the hypotheses in this field lack solid experimental evidence that proposed by Mills and Jobsis (1972) merits consideration. These authors described the occurrence of a low affinity cytochrome (cytochrome a_3) within the carotid body by exclusion and considered it to be located in the Type II cells. The essentials of the hypothesis were that, because of the low oxygen affinity, the cytochrome in the Type II cells would be

reduced by hypoxia (PaO_2 70-90 mm/Hg) then, following the depression of oxidative metabolism in the Type II cells the release of a substance (possibly potassium) would, in turn, depolarise the sensory nerve terminals. The hypothesis is obviously very attractive but lacks experimental evidence on two fronts:

1. the location of the cytochrome is not established;

2. no one has demonstrated that low affinity cytochrome is necessary for chemoreception.

On this latter point it should be mentioned that reports of low oxygen affinity cytochromes in the carotid body are not unique.

In spite of all these claims and counter-claims the structure of the mechanism by which the carotid body transfers its information remains a mystery.

CHAPTER TWO

CHAPTER TWO

MATERIALS and METHODS

INTRODUCTION

In this thesis two types of study are reported:

1. ultrastructural, stereological and morphometric studies of the carotid body with special emphasis on the Type I cells;
2. estimation of the carotid body catecholamine content and the effect of some factors on this content.

The ultrastructural experiments were performed on both cat and rat carotid bodies, utilising similar techniques which included:

- i) the use of a new sampling method;
- ii) a large sample size - to achieve more reliable results and to overcome the problem of great variability;

The determination of the carotid body catecholamine content was performed predominantly on the rat, and only results from this species are recorded here. Apart from:

- (a) the methods used for obtaining the carotid bodies;
- (b) the conditions to which the rats were exposed prior to the removal of the carotid bodies,

the analytical methods used for the estimation of the carotid body catecholamine content were identical. A relatively large number of estimations were made both in control and in experimental studies to obtain more accurate results. The high performance (pressure) liquid chromatograph with electrochemical detection (HPLC/ED) was used throughout this study. This method is highly efficient and sensitive and made it possible to measure the catecholamine content of a single rat carotid body.

2. 1. GENERAL INFORMATION

2. 1. 1. ANIMALS

A - Cats

For morphological studies of the cat carotid body 7 adult male cats weighing 1.5 - 2 kg. have been used.

B - Rats

Different strains of rats and different sources of the same strain have been used in this work as follows (see Table 2.1):

a) Wistar/Leicester (W/L).

b) Wistar-Kyoto: these were of two types - 1. Normotensive (WKY/N).

2. Spontaneously hypertensive (SHR). These rats were obtained from I.C.I. (Manchester).

STRAIN	NO. OF RATS	WEIGHT (Gram)	BLOOD PRESSURE (mm Hg)
Sprague-Dawley (S.D.)	11	275-350	-
Leicester-Wistar (L/W)	12	300-350	-
Wistar-Kyoto (WKY)	17	200-300	90-110
* Spontaneously Hypertensive (S.H.R.)	6	180-200	170-185

Table 2.1

Showing the rats used to study the carotid body catecholamine content in different strains.

* S.H.R. rats are spontaneously hypertensive type of WKY strain.

All rats used were male.

c) Sprague Dawley (Leicester bred)

d) Sprague Dawley (Bantin&Kingman (B + K) Hull, U.K.)

The animals were housed in groups of 4 - 6 under controlled conditions of light (14 hours light per day), temperature (21°C) and humidity (40%). They were fed balanced rat chow and drank tap water; both were available ad libitum.

2.1.2. ANAESTHETIC REGIMES

A. Anaesthetic regime for rat surgical procedures

The neuroleptic analgesic, immobilon (Reckitt & Colman), was used. Immobilon, consisting of etorphine 0.037 mg/ml, levomepromazine 9 mg/ml, was used diluted 1+1 with water for injection. At this concentration 1 ml/kg (i.e. 0.018 mg etorphine, 4.5 mg levomepromazine) injected intramuscularly produced adequate anaesthesia for 30 minutes.

The beauty of this anaesthetic regime is the ease with which it may be reversed by the injection of idprenorphine (Revivon, Reckitt & Colman) at a dosage of 0.07 mgm/kg i.m. Whilst the anaesthesia is reversed the analgesic effects apparently remain for several hours.

B-Anaesthetic regime for acute experiments in rats

a) Sagatal (Pentobarbitone sodium 60 mg/ml, May & Baker, Ltd.) was used in a dosage of 40 mg/kg intraperitoneally. The levels of anaesthesia produced were erratic, some animals remaining conscious after doses of 100 mgm/kg. The use of pentobarbitone was discontinued after three or four experiments.

b) Ethyl carbamate (urethane) (BDH Chemicals Ltd.) was the most frequently used anaesthetic, given in a dose of 1.25 g/kg i.p.

C - Anaesthetic regime for experiments in cats

Cats were anaesthetised with pentobarbitone sodium (40 mg/kg intra-peritoneally) or, alternatively, anaesthesia was induced with halothane and then maintained with chloralose/urethane (40/100 mg/kg). In some experiments ketamine and chloralose/urethane (40/100 mg/kg) were used as anaesthetic. Additional anaesthetic (0.15 ml/kg. 0.1%) was administered via a venous cannula as required.

2. 1. 3. ROUTINE PREPARATION OF ANIMALS FOR ACUTE EXPERIMENT

After induction of anaesthesia (see above), a tracheal cannula was inserted; in cats this was followed by cannulation of the femoral artery and vein. In all cat experiments the carotid sinus nerve was identified on one side and sectioned. The identity of the sectioned nerve was checked by observing the transient increase in blood pressure associated with cutting the sinus nerve. Sinus nerve section was performed in only eight

rats.

Immediately following nerve section the animals were ventilated with the test gas mixture for varying periods. In the ultrastructural study the test gases were 10% and 100% O₂, in the biochemical study they were 5% and 10% O₂ in N₂, 100% O₂ and 5% CO₂. Ventilation was via either a Harvard small animal respirator (model 666) or a Palmer Ideal pump. In cats arterial blood gas tensions were analysed in samples withdrawn from the femoral arterial cannula. In rats a separate series of experiments was performed in animals of the same weight to establish appropriate ventilation parameters; the values for blood chemistry are illustrated in the 'Results' section.

At the end of the test ventilation period the animals were prepared either for ultrastructural or biochemical study.

2. 2. ULTRASTRUCTURAL METHODS

2. 2. 1. PREPARATION OF SAMPLES FOR ULTRASTRUCTURAL ANALYSIS

A. Fixation of Tissue

The preparation of carotid bodies for electron microscopic studies begins with perfusion with fixative in situ. The fixative used was that of McDonald and Mitchell (1975a,b) and McDonald (1977c) which is a modification of the fixative devised by Peracchia and Mittler (1972), in some experiments 3% glutaraldehyde/1% paraformaldehyde in phosphate buffer were used.

The rats were perfused with fixative from a tube inserted into the left ventricle, blood and fixative being drained from the right atrium through another incision. The cats were perfused through a cannula inserted into the common carotid arteries immediately prior to perfusion. In the rat we perfused with two aldehyde fixatives (A and B) for 5 minutes each; 150 ml. of fixative A (at a pressure of 100 - 120 torr) followed by 50 ml of fixative B (at a pressure of less than 100 torr). Fixative A is a mixture of 3% gluteraldehyde and 10.75% hydrogen peroxide in 0.1M phosphate buffer. Solution B consists of 3% gluteraldehyde in the same buffer. Alternatively 200 ml. of the glutaraldehyde/paraformaldehyde solution was perfused.

The cat carotid bodies were perfused with 100 ml. of Solution A and 300 - 400 ml. of Solution B. Alternatively, only Solution B was used.

Following perfusion the carotid bodies were left in situ for 45 - 60 minutes, then dissected free and placed in primary fixative for a further 1 - 8 hours; they were post-fixed for 1 hour in 2% osmium tetroxide in phosphate buffer and then processed by standard techniques for embedding in Araldite or Epon.

B. Processing of Tissue for Electron Microscopy

Whole carotid bodies were embedded in a single block (for fixation and embedding see Appendix 1).

The carotid body tissue was located in the resin block using 2 μm . thick sections stained in toluidine blue. Sections were removed until a reasonable area of carotid body was available for study.

Ultrathin sections were prepared using a Reichart OMU3 ultra-microtome and mounted on single slot formvar/carbon-coated grids. An interval of 10 - 15 μm was allowed between each ribbon of sections. This procedure was repeated until sufficient levels had been obtained (6 - 12 such ribbons were used). The sections were stained in 10% methanolic uranyl acetate followed by lead citrate (see Appendix 1) and examined in either a JEOL 100S or JEOL 100 CX electron microscope.

2. 2. 2. PROCEDURES FOR STEREOLOGICAL AND MORPHOMETRIC ANALYSIS

A. A General Introduction to Stereology

Stereology is a method whereby three-dimensional information about a structure can be derived from two-dimensional sections of that structure. Stereology first gained recognition in geology and metallurgy and has only relatively recently been applied to biomorphological problems with some applications touching on cytology.

In biology stereology can be used to study the relative proportions of different tissues in organs or the changes in the content of intracellular organelles induced by experimental

procedures. It presents this information numerically, rather than purely descriptively, and thus permits an objective correlation of structural properties with quantitative physiological and biochemical data.

Stereology may be used as an adjunct to other quantitative methods, e.g. reconstruction from serial sections and serial section cinematography. In its own right, however, it is a powerful alternative approach which allows a comprehensive analysis of larger samples in a relatively short time.

In 1847 the geologist Delesse developed the fundamental relationship which provided the basis for all morphometric and stereologic study and which is now generally named the Delesse principle. This principle states that the planimetric fraction of a section occupied by sections of a given component corresponds to the fraction of the tissue volume occupied by this component (Weibel et al., 1966; Weibel, 1969). (In other words, the area density of profiles on a section (A_a) is, on average, equal to the volume density (V_v). Therefore $A_a = V_v$. This allows us to compare component volume densities by a variety of morphometric methods (e.g. measurement of transectional areas, linear intercept or point densities)).

There are several stereological techniques, each often particularly suited to a certain task; they include point analysis, line analysis, planimetry and paper weighing. The results derived from a single micrograph using each method in turn are surprisingly consistent. A full discussion is provided

by Williams (1977).

B. Sampling Techniques

Basic stereological procedures begin with an assumption of random arrangement of parts within a sample. As well as being representative, the sample should be large enough to ensure reasonable levels of accuracy for the various parameters being estimated.

The minimum sample size that must be taken to yield an accurate estimation of a component can be determined by the use of a progressive mean technique (Williams, 1977). This technique involves expressing the cumulative means as percentages of the final mean. The number of micrographs required to bring the cumulative mean permanently within 10% of the final mean is designated as the minimum sample size (MSS). Increasing the sample size obviously leads to a decrease in the errors of the measurement. In any stereological study the number of micrographs required to achieve a statistically acceptable result is inversely related to the size of the component being measured.

Clearly the fundamental issue in this type of study is to ensure that a representative sample of tissue is studied. This is usually accomplished by analysing random fields. The usual method of randomising electron micrographs relies on producing micrographs from specific areas of each grid square (see Williams, 1977). Such a technique is of little use in the study of small organs, such as the carotid body, as a complete analysis

cannot be achieved from a single organ. To this end a random sampling technique, which would yield more information per section, was developed in our laboratory (Ballard et al., 1982).

The JEOL 100S and 100 CX electron microscopes incorporate a field position indicator, thus enabling the operator to accurately locate and, if need be, return to a given point on a section.

This device consists of a fluorescent dot behind a 4 cm. diameter calibrated (in mm^2) circular screen which represents the total field of view available in a single grid. As the section is moved in the electron beam the fluorescent marker dot moves correspondingly.

Paired random numbers were generated between 0 and +4 in a computer, -2 was then added to each number to give a range between -2 and +2. These pairs were then used as co-ordinates for random points in a square 4 cm. x 4 cm.

The points falling outside a 4 cm. diameter circle were then eliminated. Each co-ordinate was squared (in order to remove negative values) and then added to the other member of the pair, e.g.

	X	Y
(1)	a	b
(2)	a^2	b^2
(3)	$a^2 + b^2$	

If $a^2 + b^2$ was greater than the square of the radius of the circle then the corresponding pair of co-ordinates was excluded.

140 points were then plotted on a 20 cm. diameter circle, drawn on graph paper using 'Letraset' dots. This drawing was then photographically reduced to a 4 cm. diameter positive image on acetate film (Figure 2.1). The graticule pattern on the graph paper was excluded at this point using a colour filter on the camera. This positive image was then fixed with sticky tape over the field positive indicator. Before using the random sample technique a piece of plain acetate was fixed over the positive image. The borders of section under examination were then plotted with a fine-tipped nylon pen, thus delineating the area of the section. Each random point within this delineated area was then aligned with the fluorescent dot and the tissue within the camera field photographed at the required magnification. In the Type I cell study the procedure was slightly modified. Here the nearest Type I cell to the random point was photographed as frequently a point and a Type I cell did not coincide. If a Type I cell was not of sufficient quality (e.g if it was contaminated by stain, or covered by a crease), it was omitted; another cell was NOT selected to take its place.

C. Techniques for Type I Cell Stereological Analysis

The Type I cells were chosen according to the random sampling technique (described above). The cells were then photographed at a medium power magnification of x10,000 and x10,500 (depending upon whether a JEOL 100S or JEOL 100 CX

FIGURE 2.1 POSITIVE IMAGE OF THE RANDOM SAMPLER ($\times 3$)

FIGURE 2.2 SHOWS EXAMPLE OF THE CUMULATIVE MEAN TECHNIQUE
IN THIS CASE:

MEAN OF OBSERVATION = 9.34

STANDARD ERROR OF MEAN = 1.31

μ = 9.34 \pm 2.57

(Where μ , the true mean has 95% possibility of
lying in the range $x \pm 1.96 \times 5.6$)

The values are within 10% confidence limits for
77.3% of the final values

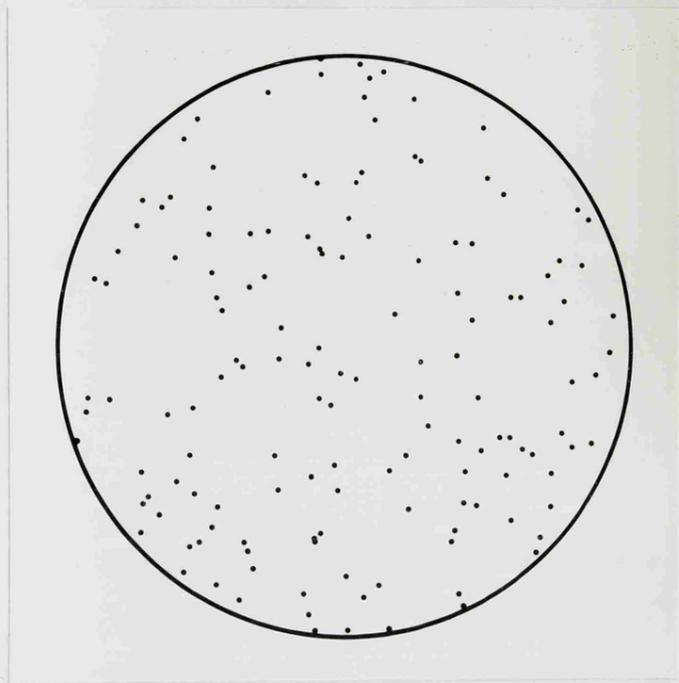


FIGURE 2.1

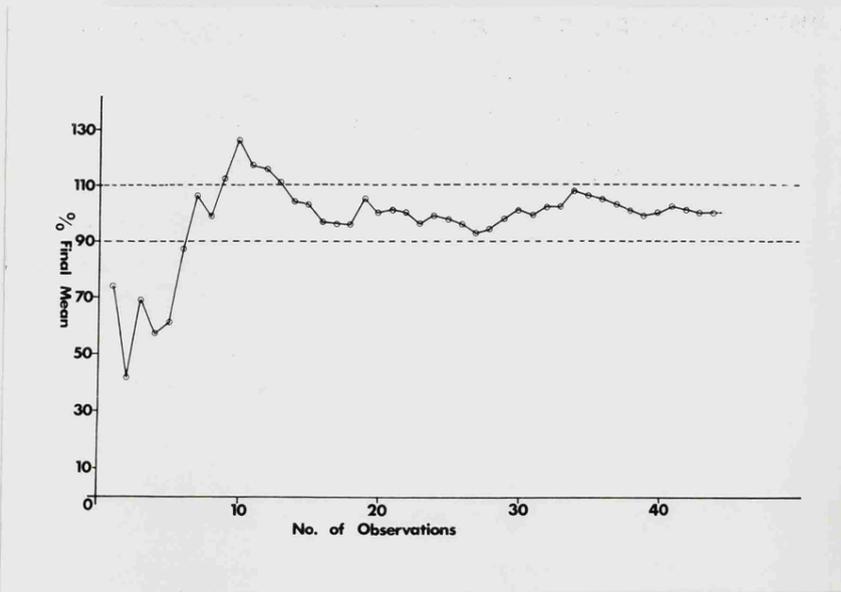


FIGURE 2.2

electron microscope was being used). At these magnifications 1 - 4 photographs were taken to cover all the Type I cells. The negatives were printed at a x3 enlargement, thus giving a final magnification of x30,000 or x31,500.

The photographs of each Type I cell were then montaged and a 'points counting' method using a 0.5 cm² graticule marked on a plain acetate sheet was employed. The counts from each feature were made using a MOP AMO2 (Kontron Ltd.) Image Analysis Unit linked on line to an APPLE II microprocessor. The number of intersections occurring over the entire cell, its nucleus, all contained mitochondria and electron dense cored vesicles were determined.

After the absolute count the values were expressed as Vv% ratios for each individual cell on line. A cumulative mean was then calculated for each component, the last value being the actual mean for each component (see Fig. 2.2).

The minimum sample sizes required to obtain satisfactory values were 27 +/- 2 cells in the cat and 30 +/- 2 cells in the rat.

The carotid bodies used for the stereological analysis were obtained from cats and rats, ventilated with 10% O₂ in N₂ and 100% O₂, whose sinus nerves had either been cut or left intact (see Table 2.2, 2.3).

D. Techniques for Type I Cell Morphometric Analysis

BLOCK NUMBER	GAS MIXTURE	STATE OF INNERVATION (C.S.N.)
HCB 5	10% O ₂ in N ₂ for 30'	CUT
HCB 6	10% O ₂ in N ₂ for 30'	INTACT
HCB 19	100% O ₂ for 30'	INTACT
HCB 20	100% O ₂ for 30'	CUT
HCB 21	100% O ₂ for 30'	CUT
HCB 22	100% O ₂ for 30'	INTACT
HCB 23	100% O ₂ for 30'	INTACT
HCB 24	100% O ₂ for 30'	CUT
HCB 41	10% O ₂ in N ₂ for 30'	INTACT
HCB 46	10% O ₂ in N ₂ for 30'	INTACT

Table 2.2

Showing cat carotid bodies used for stereological and morphometric analysis, giving the gas mixtures used and state of innervation.

ANIMAL	GAS MIXTURE	NERVE SECTION
1	100% O ₂ for 30'	None
2	100% O ₂ for 30'	None
3	100% O ₂ for 30'	None
4	100% O ₂ for 30'	None
6	100% O ₂ for 30'	None
11	100% O ₂ for 30'	None
8	10% O ₂ in N ₂ for 30'	LHS Sinus Nerve Sectioned.
9	10% O ₂ in N ₂ for 30'	RHS Sinus Nerve Sectioned.

Table 2.3

Showing the rats used for the stereological and morphometric studies of Type I cells and the experiments performed upon them giving gas mixture used and state of the innervation.

All animals used were males 275-350 grams each.

The new random sampling technique was used to select the Type I cells included in this study. The cells were photographed at an initial magnification of x2,100, then at a magnification of x21,000; 4 - 8 photographs were taken in order to cover all the cells. The negatives were printed at a x3 enlargement thereby giving a final magnification of x6,300 and x63,000. The boundaries of each Type I cell and its nucleus were marked by pen in the low magnification photograph and the high magnification photographs of each cell were montaged.

The measurements and analysis were carried out using a MOP AMO2 Image Analysis Unit linked to an APPLE II computer equipped with a specific program.

The area and nucleus of each cell was measured from, first, the low magnification photograph and then from the high magnification montage; the total number of electron dense cored vesicles were counted and the diameters of only those with clearly defined membranes were measured.

In the rat Type I cells the cell and its nuclear area were measured and the total number of electron dense cored vesicles calculated from prints with final magnifications of x30,000 and x31,500, the differences in the magnifications being adjusted by the computer. The diameters of the electron dense cored vesicles were measured from negatives with a magnification of x10,000 and x10,500. The method employed in this case involved the negatives being montaged, the Type I cells were marked with a pen and the

diameters of the EDCVs were measured directly from the negatives with the aid of a printing enlarger and a MOP AMO2 Image Analysis Unit at a final magnification of x60,000. From the APPLE II computer the following Type I cell measurements were obtained:

1. total area of the cell
2. the area of the nucleus
3. the area of the cytoplasm
4. total number of EDCVs/cell
5. number of EDCVs measured (for diameter)
6. mean of EDCV diameter (+/- S.D.)
7. median of EDCV (+/- S.D.)
8. EDCV density (number of EDCVs per μm^2 of cytoplasm)

2. 3. CATECHOLAMINE STUDIES

2. 3. 1. METHODS FOR CAROTID BODY REMOVAL

The rat carotid bodies used in the biochemical studies in this work were removed under one of the following procedures:

1. under anaesthesia
2. after cervical dislocation (C.D.)
3. after focused microwave irradiation
4. after a combination of 1 & 3
5. after a combination of 2 & 3.

This variety of techniques was used because it is well known that post-mortem metabolism of tissue catecholamines is rapid. The rates of post-mortem amine loss and accumulation of metabolites have been shown to depend on the surface area of tissue exposed to air, as well as on the time and temperature (Carlesson et al., 1974; Faiman et al., 1973; Sloviter & Conner, 1977).

To avoid the time factor, microwave irradiation could be used to inactivate the tissue enzymes in an attempt to obtain values for dopamine and noradrenaline levels which are consistent with the true endogenous concentrations (Stavinho et al 1970; Schmidt et al., 1971, 1972) Medina et al., 1974; Sharples &

Brown, 1978).

In this work focused microwave irradiation (Microwave oven, Elnode Ltd., Luton) has been used as one method to kill the rats used for catecholamine determination. It was found that 20 - 30 seconds exposure to a magnetron output of 2,500 watts (wave length 12.2 cm. and frequency of 2450 MHz) produced complete inactivation of phosphodiesterase. The animals were left to cool in crushed ice before dissecting the carotid bodies. It must be emphasised that death of the animals ensues within 5 msec. of switching on the apparatus and the rats were anaesthetised with urethane to prevent animal movement and stress factors during exposure to radiation. In the fifth manoeuvre the rats were killed by C.D. before irradiation.

These different methods were employed to establish the preferred way of removing the carotid body which produced minimum effects on the catecholamine content (Chapter 6).

2. 3. 2. SURGICAL PROCEDURES

A. Removal of the Carotid Body

The technique for removing the carotid body was essentially similar in all cases. The anatomy of the region is illustrated in Figure 2.3.

The neck was opened with a midline incision and the common

FIGURE 2.3 SELF EXPLANATORY DIAGRAM OF THE ANATOMY OF THE
CAROTID BODY REGION IN THE CAT

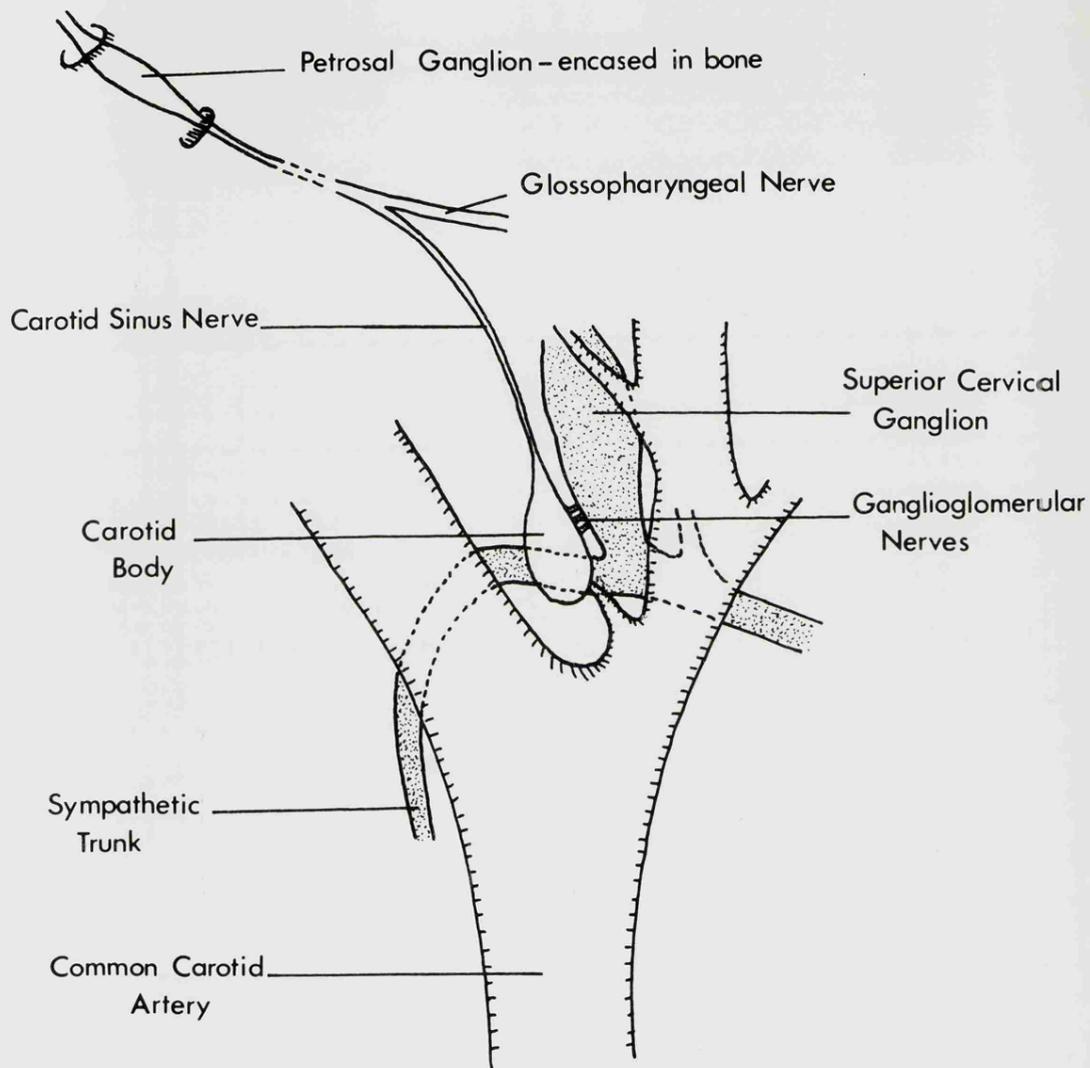


FIGURE 2.3

carotid artery and its bifurcation region exposed. A bipolar coagulator (Micro Bipolar Coagulator, Downs Surgical Ltd. Ser. No. 411) was used to ensure a blood-free field. The superior cervical ganglion was removed in order to prevent contamination of the carotid body with ganglionic tissue. The carotid body, which lies immediately adjacent to the internal carotid artery, was then removed together with adjacent connective tissue. It must be stressed that no attempt was made to isolate the carotid body from this tissue as this would greatly have increased dissection time. As the blood supply to the organ is almost certainly damaged during this procedure it is important that dissection time be kept to a minimum (c 5 minutes).

B. Surgical Sympathectomy (ganglionectomy) with Recovery

The term 'surgical sympathectomy' has been applied to both transection of post-ganglionic sympathetic nerves and the removal of the corresponding ganglion (Thoenen, 1972). In this work unilateral sympathectomy was performed as follows:

1. anaesthesia was induced as described above;
2. the neck region was shaved and the neck opened with a mid-line incision;
3. the bifurcation of the common carotid artery on the chosen side was exposed by blunt dissection;
4. small blood vessels were cut with the aid of a microbipolar coagulator;

5. the superior cervical ganglion, which was usually located medial to the external carotid artery, was removed as follows: a) the pre-ganglionic sympathetic trunk was located and sectioned just proximal to the ganglion;

b) the ganglion was separated from surrounding tissue by blunt dissection; nerve branches were sectioned with the bipolar coagulator. Particular care was taken in sectioning the ganglioglomerular nerve in order to avoid damage to the carotid body;

c) the ganglion was finally removed by sectioning the superior post-ganglionic nerve.

The anatomy of the region is illustrated in Figure 2.3.

In some animals the right superior cervical ganglion was removed; in others, the left. The wound was sprayed with gentian violet/ oxytetracycline spray and closed with mattress stitches. During surgery the animals were maintained on a heated blanket to conserve body temperature.

2. 3. 3. CHEMICAL SYMPATHECTOMY

6-hydroxydopamine (6-OH-DA) has been shown to produce a long-lasting depletion of noradrenaline from various peripheral organs and a functional failure of transmission at sympathetic nerve endings of several organs (Laverly et al., 1965; Thoenen & Tranzer, 1968). 6-OH-DA has also been shown to behave in many

respects as a catecholamine; thus 6-OH-DA can be taken up and accumulated in the adrenergic nerve mechanism (Jonsson & Sachs, 1970,1972). Administered in a small dose, 6-OH-DA can be released upon nerve stimulation and acts as a 'false transmitter' (Thoenen & Tranzer, 1968). Initially 6-OH-DA seems to cause a displacement of endogenous NA but when a critical concentration of 6-OH-DA is reached intraneuronally rapid and severe damage to the catecholamine uptake/storage mechanism takes place and there is a subsequent degeneration of nerves (Jonsson & Sachs, 1970). 6-OH-DA causes a selective reversible degeneration of axon terminals (but not cell bodies) or peripheral adrenergic neurons in vitro and in vivo (Thoenen & Tranzer, 1968,1973).

The sensitivity to 6-OH-DA degeneration varies from fibre to fibre and from ganglion to ganglion. The time course of degeneration is also variable and may be related to the amount of 6-OH-DA which is taken into the neuron to produce a degeneration effect. Recovery time varies and probably depends on the length of the degenerated terminal fibres (Burnstock & Costa, 1975).

Although the exact molecular mechanism leading to neuronal damage and subsequent degeneration is unknown it has been shown in in vitro experiments that during auto-oxidation of 6-OH-DA several potentially toxic products are formed. These include 6-OH-DA quinones, hydrogen peroxide (H_2O_2) and several free radicals, all of which have been suggested as playing causative roles in the degeneration process (Saner & Thoenen, 1971).

In this work chemical sympathectomy was performed by giving

the rats the neurotoxin 6-OH-DA (2, 4, 5-trihydroxyphenylethylamine, hydrobromide- crystalline, Sigma, U.K. (mol. wt. 250.1)). The 6-OH-DA solution (1mg./ml in 0.9% saline containing 1mg./ml. ascorbic acid) was prepared freshly prior to injection; it was injected intravenously in a dosage of 50 mg/kg (375 μ mol/kg) via the tail vein, on days 1, 2 and 7. The controls were injected with solvent only. The animals were killed seven days following the last injection. According to Kostrzewa and Jacobowitz (1974) such a dose regime has been shown to destroy noradrenergic nerve terminals in several tissues. The efficacy of the dosing regime was checked by determining catecholamine levels in samples of the left ventricle of the heart.

2. 3. 4. CATHECHOLAMINE DETERMINATION

The high pressure liquid chromatography (HPLC) with electrochemical detection technique has been used in all the determinations of rat carotid body catecholamines.

A. Samples Preparation

The carotid bodies were dissected in the usual way (see above), each carotid body being placed in an homogenization tube containing 250 - 600 μ l. of 0.1M perchloric acid (Perchloric Acid AR 69 - 71%, Fisons Analytical Reagent) containing 0.1 mM EDTA (Diamino ethanetetra-acetic acid disodium salt, AR, Fisons). The samples were kept on crushed ice and the homogenization performed manually. The samples were then

transferred to 1.5 ml. polypropylene tubes and centrifuged in a Beckman Microfuge B at 9,700 g for 30 - 60 seconds. The supernatant was transferred to another 1.5 ml. polypropylene tube. The samples were either analysed directly or deep frozen at -40°C .

B. Chromatography

In our work for determination of carotid body catecholamines by HPLC with electrochemical detection we used for separation an Altex Model 110A (Beckman Instruments, U.S.A.) pump, connected to an Altex pump dampener (Part 110 - 140). The pump was used on a flow rate of 0.9 ml/min. and 200 - 300 psi.

The injection systems used were either Altex Universal Injection Valve (Altex 210) or the auto-sampler, Series 8000 (Varian Ltd. Instrument Group, U.S.A.). The valve can operate at a pressure of 2,000 psi. 50-100 μl samples were injected according to the loop used. Analytical columns used were the strong cation exchange columns (Partisil Column - 10 SCX, 25 cm length x 4.6 mm I.D. or VYDAC column - 10 SCX, 50 cm length x 4.6 mm I.D. - both from Anachem Ltd.) or the Altex Ultrasphere IP, 5 μm (4.6 mm I.D. x 25 cm length).

The solvent system used was a 0.5 M acetate-citrate buffer (pH 5.2) containing EDTA (ethylene diamine tetra-acetic acid) (0.1 mM) as the mobile phase (for details see Appendix 2). This buffer was used with the reverse phase ion-pair prepacked columns

but with strong cation exchange columns, the same buffer but without methanol and sodium octyl sulphate was used. HPLC solvents were all Analar grade (Fisons, Loughborough).

The electrochemical detection was performed by a model LC-4 (Bioanalytical Systems Liquid Incorporated, U.S.A.). Amperometric detector equipped with TL-3 detection electrode (Bioanalytical Systems, U.S.A.) packed with carbon paste (oil based (CP-0 BAS). The cell which contains the carbon paste detector electrode consists of a fluoro-carbon gasket (TG-2M 0.05 mm thick Gasket (B.A.S.) (U.S.A.) between two inert plastic blocks.

An applied potential of + 0.65V was maintained on the detector electrode, scale sensitivity used were either 0.5 or 1.0 nA, according to volume or sample injected (50-100 μ l).

Chromatograms were recorded on either a CR 600 (J.J. Instruments, Britain) recorder or a Perkin-Elmer Model 56 Recorder (U.S.A.), alternatively a SHIMADZU, C-RI, Chromatopack integrator was used in combination with an auto-sampler series 8000 (Varian Limited Instrument Group, U.S.A.).

C. Standard Preparations

Stock standard solutions were prepared containing 50 pM of noradrenaline, dopamine and adrenaline in 100 μ l of 0.1 M P.C.A. These were immediately frozen at -40°C in 2 ml aliquots until required. At the time of analysis one aliquot was thawed of each

catecholamine standard mixed in equimolar preparations and diluted to a final concentration of 5 pM in the injection loop volume (50 μ l for manual injection, 100 μ l for automatic injection). All material was obtained from Sigma Chemical Co., U.S.A.).

D. Analysis of Catecholamines

Analysis of samples was according to the following protocol:

1. injection of standards until a uniform peak height was achieved;
2. injection of 4 unknowns;
3. injection of standard.

By this means it was possible to check for changes in sensitivity during the analysis period.

In preliminary experiments to evaluate the utility of HPLC/ED for carotid body catecholamine determination the linearity and detection limits of the system were evaluated.

To test linearity of analyser standard samples containing 1, 2, 5 and 10 p mols of NA, AD and DA were used then the height of the peaks of the components of the standard were plotted against their concentrate in each sample. (Figure 2.4) shows the linearity of HPLC/ED we used chromatographic reproducibilities of

FIGURE 2.4 SHOWS THE LINEARITY OF THE HIGH PRESSURE LIQUID CHROMATOGRAPHY WITH ELECTRO-CHEMICAL DETECTION (HPLC/ED)

Ordinates: Peaks height of Noradrenaline (NA), Adrenaline^(AD) and Dopamine (DA) standards (nA)

Abscissa: The concentrations of the standards injected (p mol)

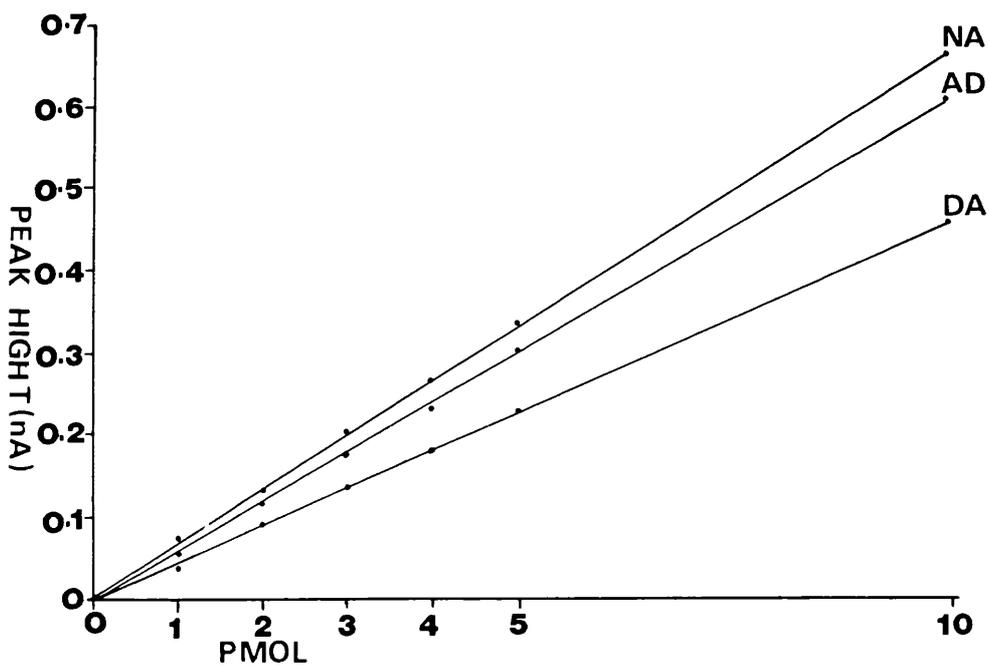


FIGURE 2.4

about 10% are adequate.

The sensitivity of this assay was 0.2 p mol for noradrenaline and 0.5 p mol for dopamine and adrenaline.

E. Calculation

External standards were run between every 4-6 samples.

Calculations were done by comparing the peak heights of NA, AD or DA samples to that of the external standard and multiplying by the dilution of the sample.

Peak height was measured rather than area because it is less influenced by overlapping peaks. In later experiments the calculations were performed on-line by the integrator.

2. 4. PHYSIOLOGICAL TECHNIQUES

The effects of breathing different gas mixtures on carotid body catecholamine contents were studied in the rat.

The animals were subjected to the different gases by two methods:

A. Spontaneous Breathing

The rats were anaesthetised with urethane (1.25 g/kg i.p.) and placed in an environmental chamber which is connected to the

required atmosphere. The gas flow was adequate to maintain an atmosphere of required composition as checked by analysis of the chamber gas and animal blood chemistry. The chamber lid was left open for rats breathing room air (control). In separate experiments blood gas tensions and pH analysis was carried out each condition by placing a cannula into one common carotid artery. The rats were then exposed to the experimental conditions for the appropriate period of time (30 or 60 minutes) and a blood sample was taken for analysis (see below for analysis). These rats were not used for catecholamine studies. As a control the blood sample was taken immediately after rats were anaesthetised.

B. Artificial Ventilation

The animals were anaesthetised and a tracheal cannula inserted as described above; animals were then ventilated with the test gas (either air, 10% O₂, 5% O₂, 100% O₂ or 5% CO₂). In preliminary experiments for each gas mixture the required pump parameters were determined by measuring arterial blood gases in animals of similar weight (see Table 7.1). The carotid bodies from these animals were not used for analysis. During all the experiments the animals were kept warm by a rat warmer electric pad and rectal temperature kept at 35-36°C.

Following the 1st ventilation period the carotid bodies were removed as described above.

Blood Gas Analysis

Blood samples were taken into heparinized tubes and sealed with wax. The samples were then analysed on a B.M.S. Mk 2 micro system (Radiometer Copenhagen) for $P_{a}O_2$, $P_{a}CO_2$ and pH_a .

CHAPTER THREE

CHAPTER THREE

MORPHOLOGICAL STUDIES OF THE CAT AND RAT CAROTID BODIES

The cat and rat carotid bodies are similar in their general morphological appearance; the light microscopical and electron microscopical findings will therefore be described here in general terms. These results are in broad agreement with previous descriptions of the carotid body (see Verna, 1979).

3.1. LIGHT MICROSCOPY OF THE CAROTID BODY

In semi-thin sections of carotid body tissue embedded in epoxy resins and stained with toluidine blue, the carotid body is seen to be enveloped by a connective tissue capsule (see Figure 3.1) and contain broadly three main components; (a) specific tissue (glomoid), (b) blood vessels, (c) stratum nervosum (see Figure 3.2).

The specific tissue consists of Type I and Type II cells arranged in irregular cords or lobules. The former are usually polygonal in shape with large round nuclei and each group is partially surrounded by Type II cells (see Figure 3.3); the latter are often compared with the Schwann (or Satellite) cells in the peripheral nervous system. In light micrographs Type I and Type II cells can ^{be} distinguished on the basis of nuclear morphology (Figure 3.4).

FIGURE 3.1 L.M. GLOMUS TISSUE IN RAT CAROTID BODY (x 75)

Ct - Connective tissue

C - Capillary

FIGURE 3.2 L.M. GLOMUS TISSUE IN RAT CAROTID BODY (x 300)

C - Capillary

S - Stratum nervosum

g - Glomoid

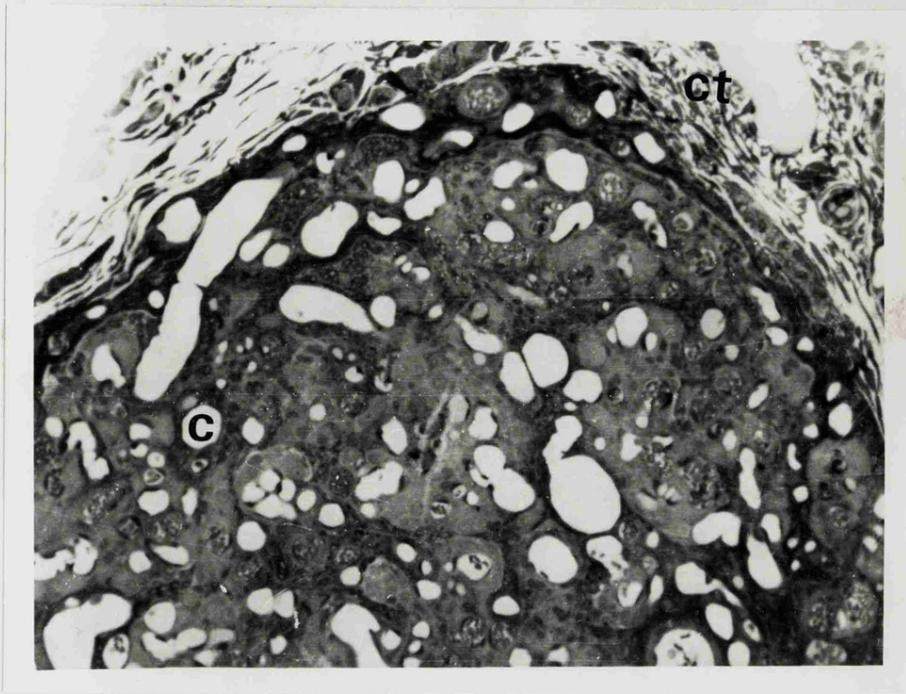


FIGURE 3.1

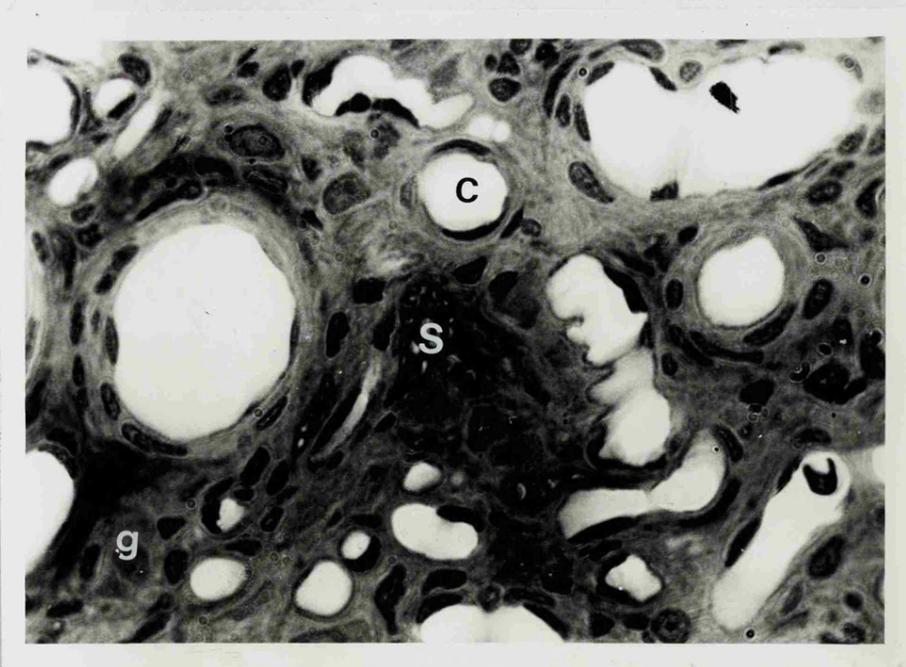


FIGURE 3.2

FIGURE 3.3 L.M. GLOMUS TISSUE IN RAT CAROTID BODY (x 400)

- I - Type I cell
- II - Type II cell
- C - Capillary

FIGURE 3.4 L.M. GLOMUS TISSUE IN RAT CAROTID BODY (x 300)

- I - Type I cell nucleus
- II - Type II cell nucleus
- C - Capillary
- S - Stratum nervosum

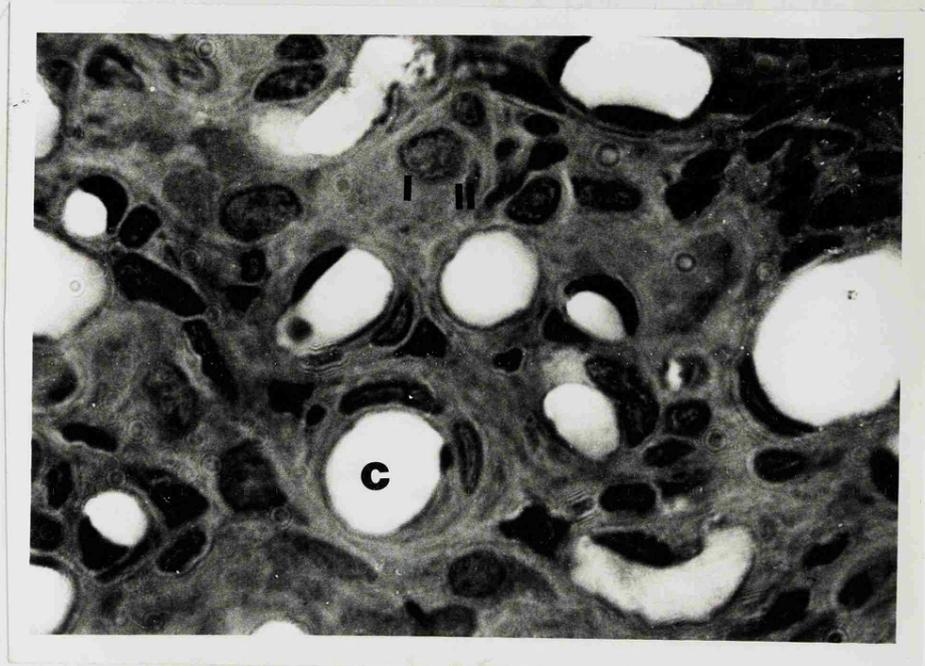


FIGURE 3.3

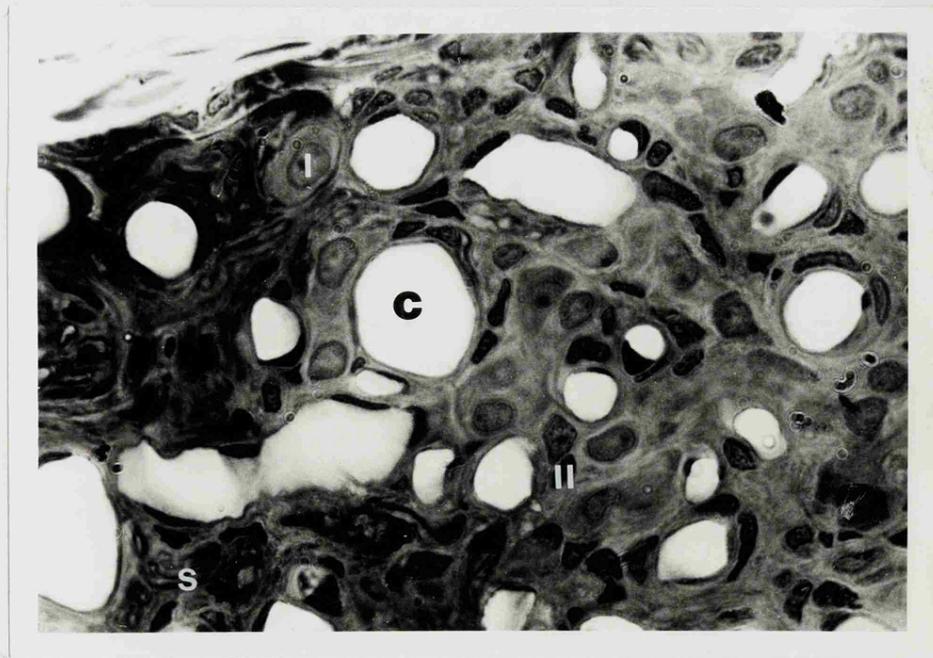


FIGURE 3.4

Blood vessels are frequently observed in the interlobular connective tissue (see Figure 3.5). Small arteries and veins occur throughout the carotid body but, within the specific tissue, the predominant vascular elements are the capillaries.

The stratum nervosum comprises connective tissue, myelinated and unmyelinated nerve fibres, fibroblasts, mast cells etc. Myelinated nerve fibres occur throughout the organ but are more common on the periphery, especially at the neural pole. In the rat carotid body it is said that the sinus nerve fibres supplying the carotid sinus pass through the carotid body.

3. 2. THE ULTRASTRUCTURE OF THE CAT AND RAT CAROTID BODY

3.2.1. GENERAL APPEARANCE

The basic morphology of the carotid body is similar in the cat and rat, except that the rat carotid body appears to have more specific tissue than that of the cat.

The lobules or clusters of the carotid body consist of 4-5 Type I cells, partially surrounded by the cell body and process of a Type II cell (Figure 3.6); the lobules are separated by connective tissue, blood vessels and nerve fibres. At the electron microscopical level the capillaries are seen to be fenestrated and surrounded by subendothelial space of varying width which separates the Type I cells from

FIGURE 3.5 L.M. GLOMUS TISSUE IN RAT CAROTID BODY (x 150)

Lv - Large veins on carotid body surface

g - Glomoid

C - Capillary

A - Adipose tissue

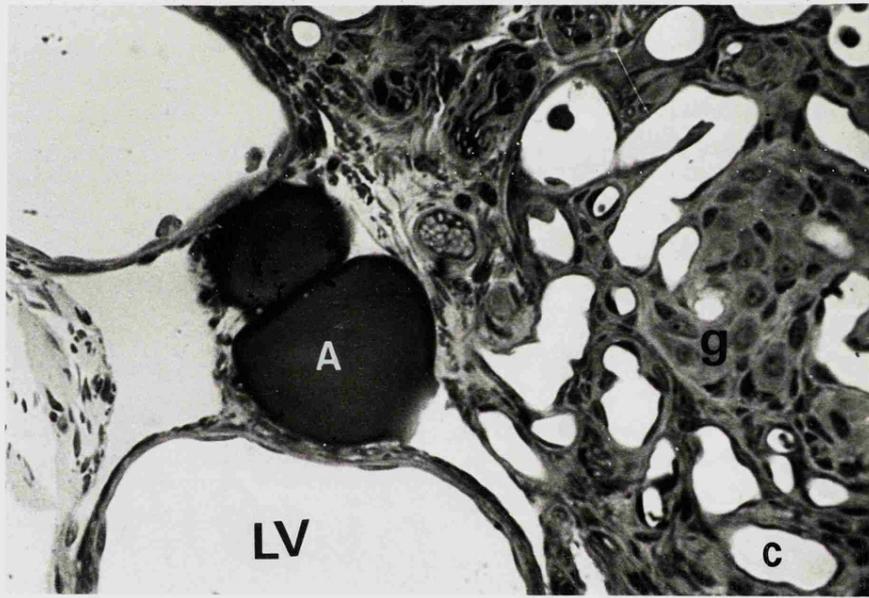


FIGURE 3.5

FIGURE 3.6 LOW POWER (x 2,100) ELECTRON MICROGRAPH OF GLOMUS
TISSUE IN CAT CAROTID BODY

I - Type I cell

II - Type II cell

uf - unmyelinated nerve fibre

mf - myelinated nerve fibre

E - Nerve ending in contact with Type I cell

Ca - Capillary

Co - Collagen

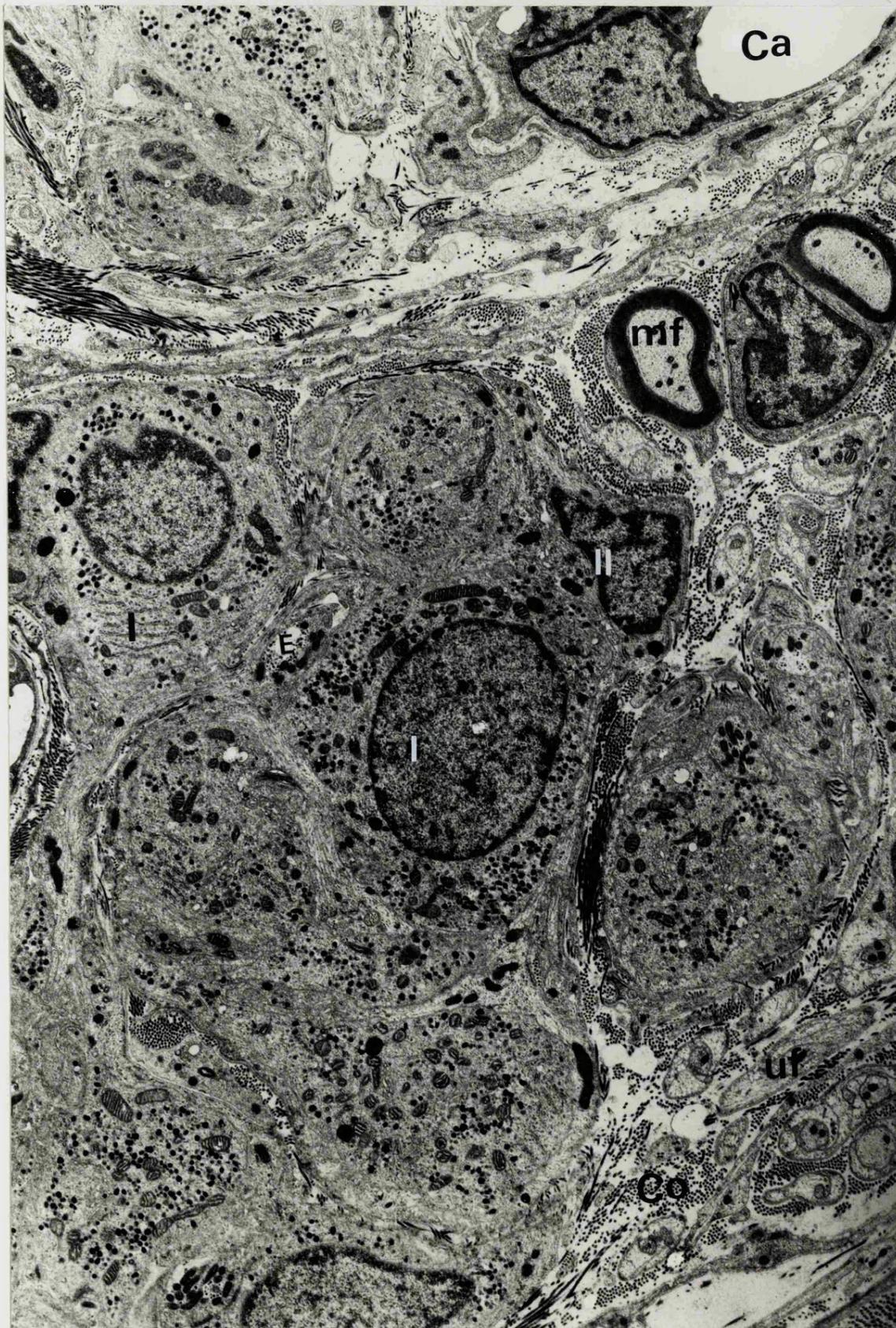


FIGURE 3.6

the vessel walls (see below and Fig.3.12).

3.2.2. THE SPECIFIC TISSUES

A. Type I Cells

The ultrastructure of the Type I and Type II cells is illustrated in Figures 3.7 - 3.11. The Type I cells are irregularly elongated and have a roughly spindle-shaped cell body with one or more cell processes. These processes are larger and occur more frequently in the rat Type I cells. The cell body itself is approximately 10 μm in diameter, while its processes may be up to 15 μm long in the cat and several times this length in the rat(see Fig. 3.7A-3.7D).

Nuclei are of a finely granulated appearance with an outer border of high electron density. Each contains a single prominent nucleolus. There are numerous nuclear pores.

The cells have a profile typical of metabolically active secretory cells. They have numerous small mitochondria (length 1 μm , width 0.3 μm) with closely packed transverse or longitudinal cristae extending without break across the mitochondrion; a fairly well-developed Golgi body; numerous small clear vesicles (some of which are coated) and an extensive lamellar arrangement of rough endoplasmic reticulum. Ribosomes also occur in free regular groupings or clusters but at least half are associated with endoplasmic reticulum. Cilia are found within the Type I cells; this type

FIGURE 3.7. A ELECTRON MICROGRAPH OF GLOMUS TISSUE IN RAT
CAROTID BODY (x 5,600)

I - Type I cell
II - Type II cell
NI - Type I cell nucleus
m - mitochondria
v - electron dense-cored vesicles

FIGURE 3.7. B ELECTRON MICROGRAPH OF TYPE I CELL IN CAT
CAROTID BODY (x 21,000)

N - Nucleus
Cy - Cytoplasm
m - mitochondria
v - electron dense-cored vesicles
ER - Rough surfaced endoplasmic reticulum
r - ribosomes

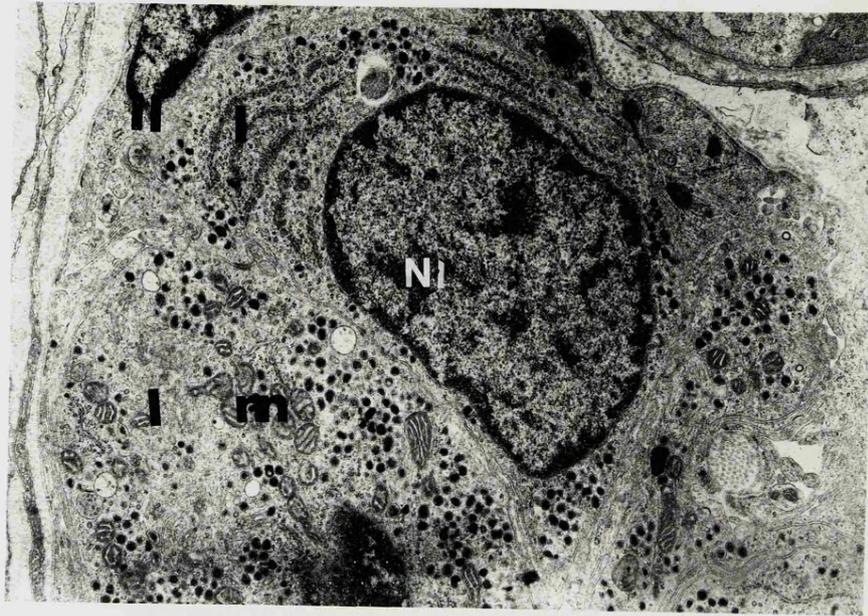


FIGURE 3.7. A

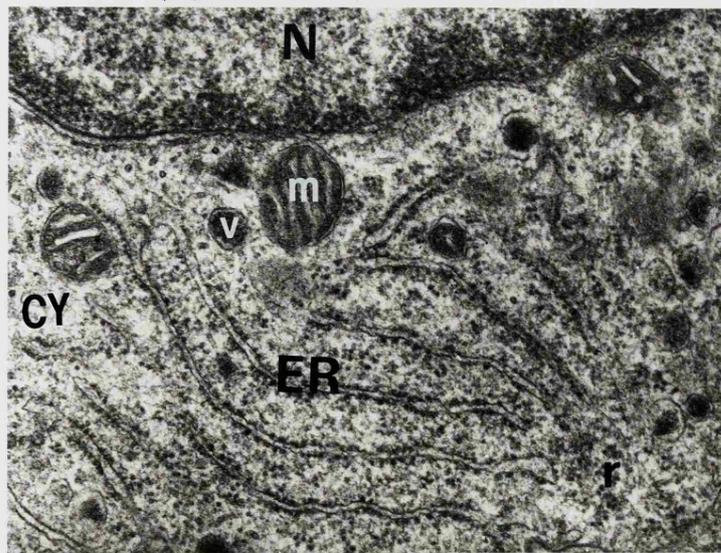


FIGURE 3.7. B

FIGURE 3.7. C ELECTRON MICROGRAPH OF TYPE I CELL IN CAT
CAROTID BODY (x 21,000)

I - Type I cell
N - Nucleus
m - mitochondria
v - electron dense-cored vesicles
SR - Smooth surfaced endoplasmic reticulum

FIGURE 3.7. D ELECTRON MICROGRAPH OF TYPE I CELL IN RAT
CAROTID BODY (x 13,000)

Cy - Cytoplasm
N - Nucleus
C - Cilium
m - mitochondria
g - Golgi apparatus

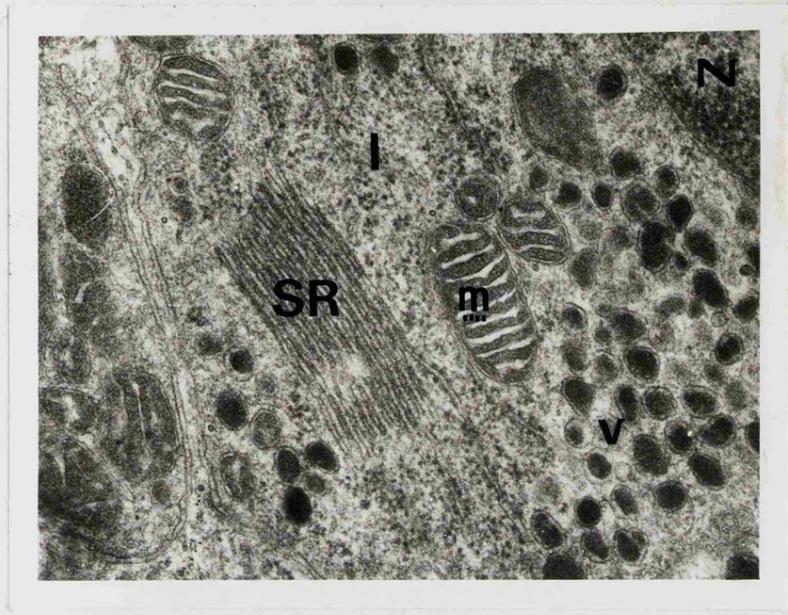


FIGURE 3.7. C

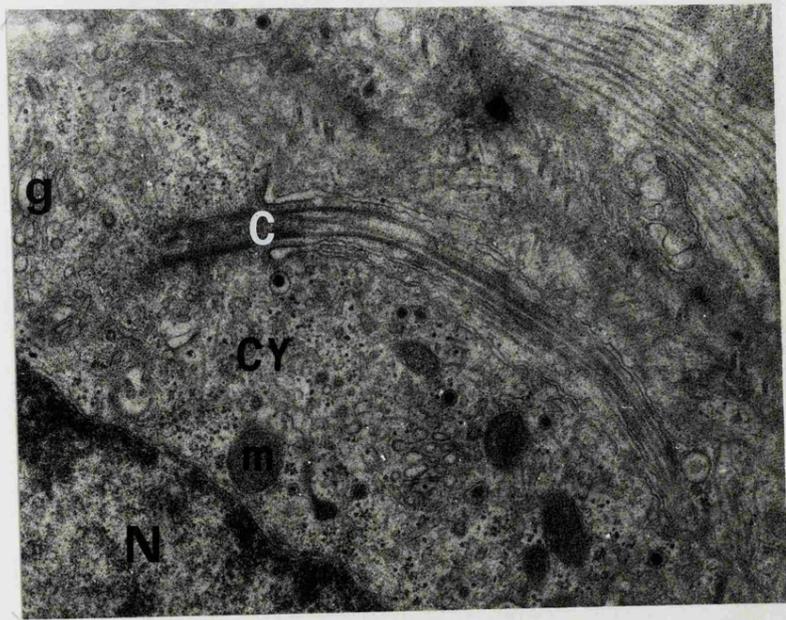


FIGURE 3.7. D

of cilium structure has been reported at a number of different sites (see Biscoe, 1971) - their function within the carotid body is undetermined. Large lysosome-like bodies are occasionally noted in Type I cells. They contain heterogeneous material which may possibly be broken down cytoplasmic inclusions, or may reflect ageing of the cell.

The most distinctive and consistent feature of the Type I cell is its dense cored vesicles, which differ in number both from cell to cell and within various regions of a single cell. Although they may occur in regions of high and low density (Figure 3.8), there is no pattern to these accumulations. There is a wide vesicle size range, although they average about 140-160 nm in diameter in the cat, but are somewhat smaller in the rat (see Chapters 4 and 5).

The dense cored vesicles are surrounded by a thin but clearly defined membrane. The light zone, which separates the membranes from the dense core, varies in width in different vesicles; accordingly the extent to which the core fills the vesicles also varies and in some of the larger ones the core constitutes but a small fraction of the whole and is eccentric in position. 'Paired', lozenge-shaped vesicles were also observed with a central septum dividing two distinct accumulations of dense material (Figure 3.9).

Specialised junctions between cells and cell processes are rare in the cat carotid body; since the Type II cell processes are usually extensive, regions of contact between

FIGURE 3.8 LOW POWER MICROGRAPH OF GLOMUS TISSUE IN CAT CAROTID BODY ($\times 3,300$) SHOWING THE VARIABILITY OF THE DENSITY OF ELECTRON DENSE-CORED VESICLES IN DIFFERENT TYPE I CELLS AND IN DIFFERENT REGIONS OF THE SAME CELL

- I - Type I cell
- II - Type II cell
- mf - Myelinated nerve fibre
- Ca - Capillary lumen
- E - Endothelium
- v - electron dense-cored vesicles
- uf - unmyelinated nerve fibre
- e - nerve ending in contact with Type I cell



FIGURE 3.8

FIGURE 3.9 ELECTRON MICROGRAPH SHOWING "PAIRED" ELECTRON DENSE-CORED VESICLES IN TYPE I CELL CYTOPLASM IN CAT CAROTID BODY (x 63,000)

- v - electron dense-cored vesicle
- Pv - Paired electron dense-cored vesicles
- m - mitochondria
- g - Golgi apparatus
- Ly - Lysosome
- Arrow - Coated Vesicle

FIGURE 3.10 ELECTRON MICROGRAPH OF TYPE II CELL ENCLOSING UNMYELINATED NERVE FIBRES IN RAT CAROTID BODY (x 32,000)

- II - Type II cell cytoplasm
- uf - unmyelinated nerve fibre
- N - Nucleus (lobulated)
- C - Collagen
- m - Mitochondria

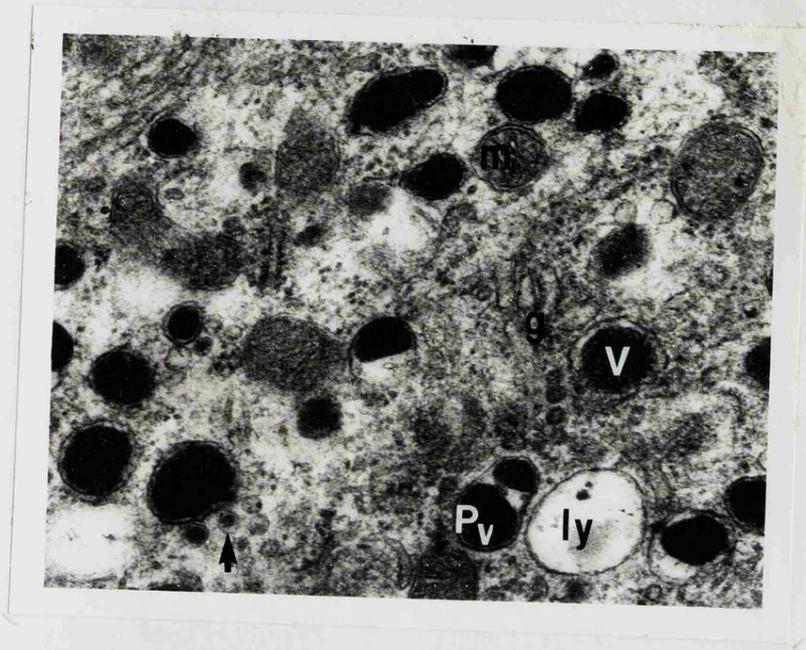


FIGURE 3.9

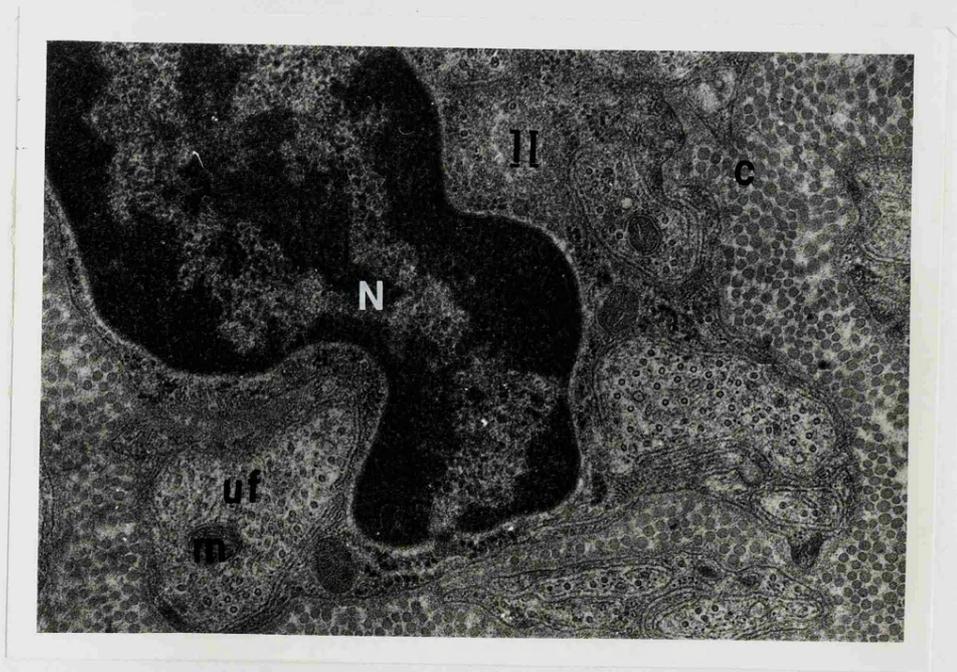


FIGURE 3.10

the Type I cells are limited and occasionally seen. In the rat this type of contact is more frequent as the Type II cell processes are surrounding, rather than penetrating, the Type I cell groups.

Although Type I cells and fenestrated capillaries are often seen in close apposition, they are separated by the basal lamina of both, the walls of the capillaries in addition to fibroblast processes and several layers of collagenous connective tissue.

B. The Type II Cell

The Type II cells, which partially invested the Type I cells, were frequently related to nerve fibres in the same manner as are nerves to Schwann cells (Figure 3.10). The nuclei were situated in the broadest segment of the cells and occasionally found to be multi-lobed; they were also more granular with less cytoplasmic inclusions than in Type I cells (see Figure 3.11).

3. 2. 3. THE BLOOD VESSELS

The blood vessels were numerous - the thin-walled capillaries were the most common vessels observed within the specific tissue, their endothelial cells contained the usual cytoplasmic constituents and prominent pinocytic vesicles. Fenestrae were found in the attenuated segments of the endothelial cells (Figure 3.12). In addition, the connective

FIGURE 3.11 ELECTRON MICROGRAPH OF TYPE II CELL AT PERIPHERY
OF A TYPE I CELL GROUP IN RAT CAROTID BODY (x10,000)

- I - Type I cell
- II - Type II cell
- C - Collagen
- m - mitochondria

FIGURE 3.12 ELECTRON MICROGRAPH OF FENESTRATED CAPILLARY
IN RAT CAROTID BODY (x 28,000)

- F - Fen^estrae
- L - Capillary's lumen
- I - Type I cell

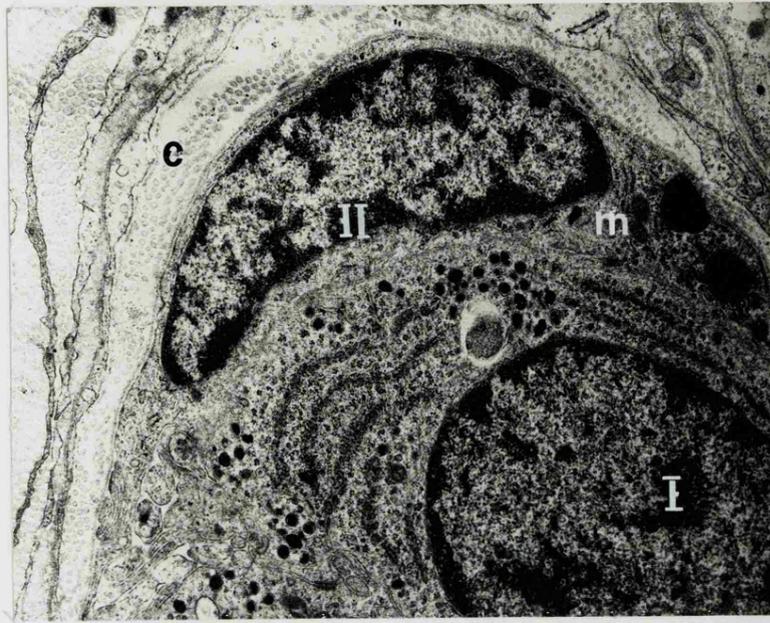


FIGURE 3.11

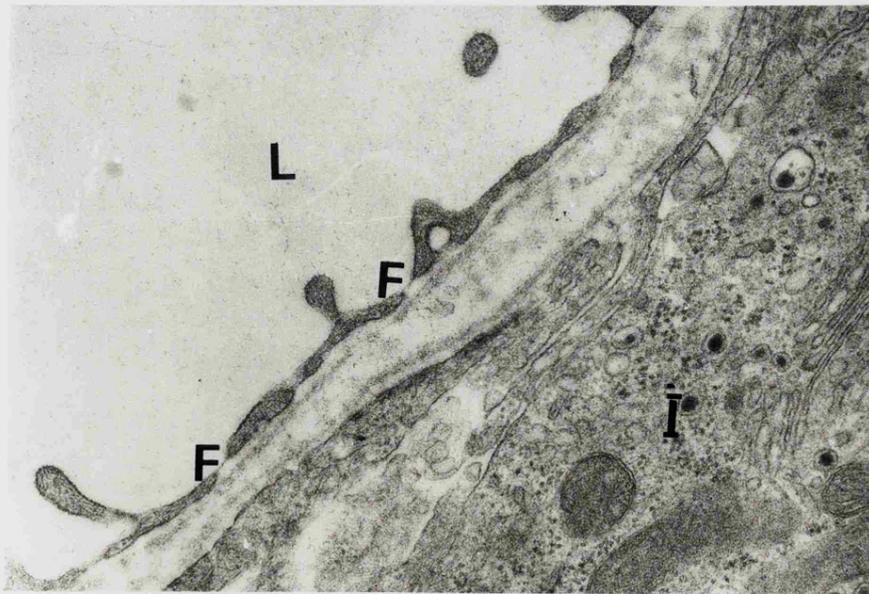


FIGURE 3.12

tissue elements surrounding the organ contain numerous large vessels (Figure 3.5). In view of recent results of Acker and O'Regan (1981), these vessels may be of extreme importance in the overall functioning of the carotid body.

3.2.4. THE NERVE FIBRES AND TYPE I CELL NERVE ENDINGS

A. Nerve Fibres

1. Unmyelinated Nerve Fibres

These fibres, more frequently observed in the rat than in the cat carotid body. They are found within the Schwann cells or Type II cells. They contained microtubules, microfilaments and mitochondria (fig. 3.10. & Fig. 3.13A).

2. Myelinated Nerve Fibres

In general these were less numerous than the unmyelinated nerve fibres, but were located at all levels of the carotid body and in greater numbers near the periphery. They were most common at the neural pole of the carotid body. Their typical ultrastructural profile is illustrated in Figure 3.13B.

In the perilobular region both myelinated and unmyelinated fibres were surrounded by a neurolemmal sheath; as they penetrate the lobule they lose their myelin sheaths.

FIGURE 3.13 A ELECTRON MICROGRAPH OF UNMYELINATED NERVE FIBRES
IN RAT CAROTID BODY (x 20,000)

uf - unmyelinated nerve fibre
m - mitochondria
C - Collagen

FIGURE 3.13 B ELECTRON MICROGRAPH OF MYELINATED NERVE FIBRE
(x 16,000)

mf - myelinated nerve fibre
M - Myeline sheath
m - mitochondria
C - Collagen
I - Type I cell

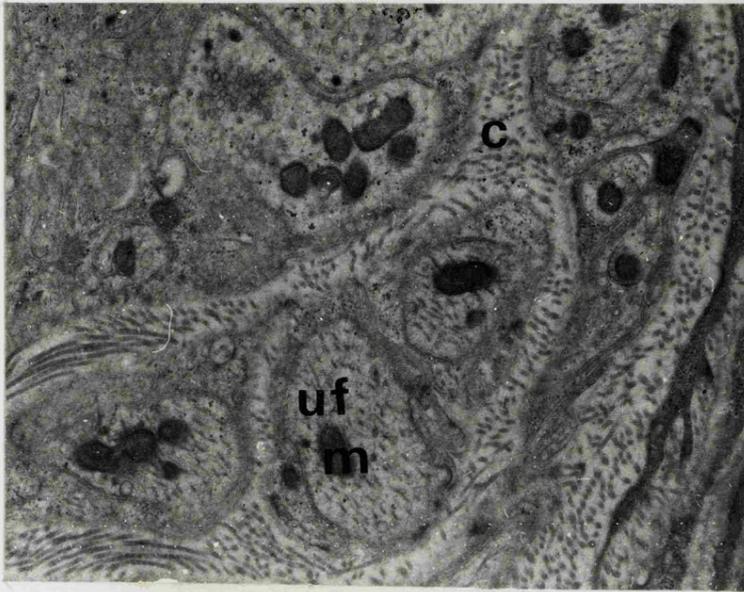


FIGURE 3.13 A

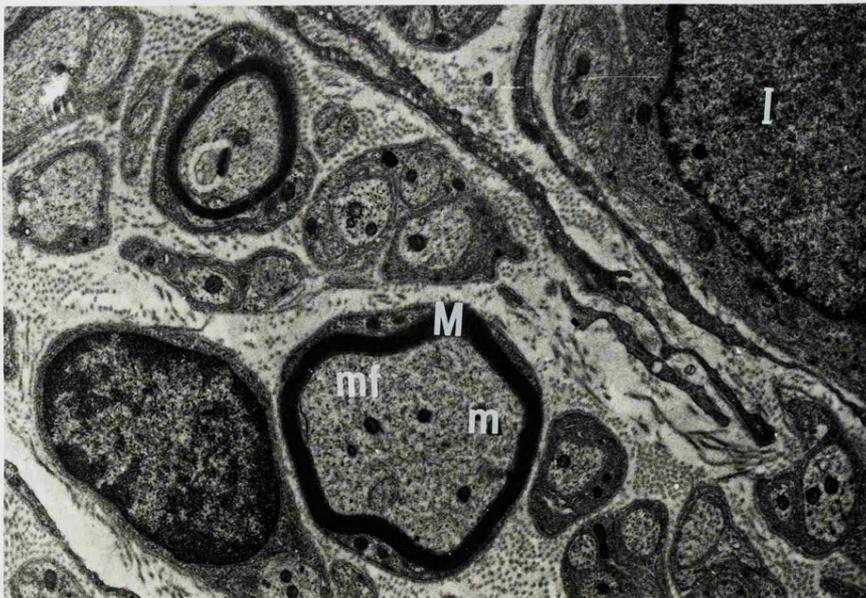


FIGURE 3.13 B

B. Nerve Endings And Type I Cells

Nerve endings were recognised and distinguished from nerve fibres passing through the Type II cells by their size, the presence of accumulations of mitochondria and small vesicles; they were distinguished from Type I cell processes by the absence of ribosomes.

The Type I cell nerve endings were characterised by their great variability in shape, size and content (Figure 3.14 shows this feature). The endings contained smaller mitochondria than found in Type I cells (0.5 μm in length, 0.15 μm in width). The small clear vesicles in nerve endings were often present in large numbers, were similar to the synaptic vesicles described at synapses in the central nervous system (Gray, 1966; see also Biscoe & Stehbens 1966) and were sometimes concentrated where the nerve endings formed specialised junctions with Type I cells (Figure 3.14A,B,&C.). The density of these synaptic vesicles and mitochondria was highly variable. Electron dense cored vesicles were also found (Figure 3.15). They were more numerous in rat than cat endings. Glycogen granules were also seen.

Based on the synaptic classification introduced by Gray the endings could be divided into three types: afferent, efferent and reciprocal (Fig. 3.16A,B, & C); however, the majority were unclassifiable. In the cat efferent endings occur most commonly, in the rat afferent nerve endings occur

FIGURE 3.14 A ELECTRON MICROGRAPH SHOWING NERVE ENDING IN CONTACT WITH TYPE I CELL IN CAT CAROTID BODY (x 28,000)

I - Type I cell
NE - Nerve ending
m - mitochondria
S - Synaptic-like vesicles

FIGURE 3.14 B ELECTRON MICROGRAPH SHOWING NERVE ENDING IN CONTACT WITH TYPE I CELL IN CAT CAROTID BODY (x 28,000)

I - Type I cell
NE - Nerve ending
m - mitochondria
g - glycogen granules
S - Synaptic-like vesicles

FIGURE 3.14 C ELECTRON MICROGRAPH SHOWING NERVE ENDING IN CONTACT WITH TYPE I CELL IN CAT CAROTID BODY (x 10,500)

I - Type I cell
NE - Nerve ending
m - mitochondria

FIGURE 3.14 A



FIGURE 3.14 B

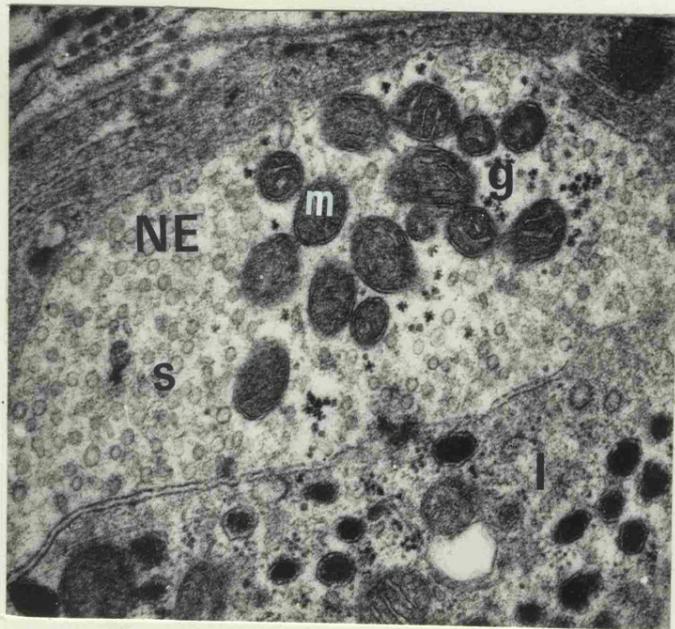


FIGURE 3.14 C

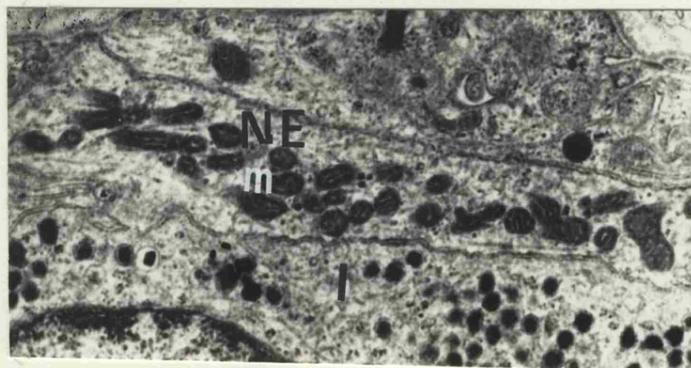


FIGURE 3.15 ELECTRON MICROGRAPH SHOWING NERVE ENDING CONTAINING
ELECTRON DENSE-CORED VESICLES IN CONTACT WITH TYPE
I CELL IN CAT CAROTID BODY (x 28,000)

I -- Type I cell
NE - Nerve ending
S - Synaptic-like vesicles
v - electron dense-cored vesicle
m - mitochondria

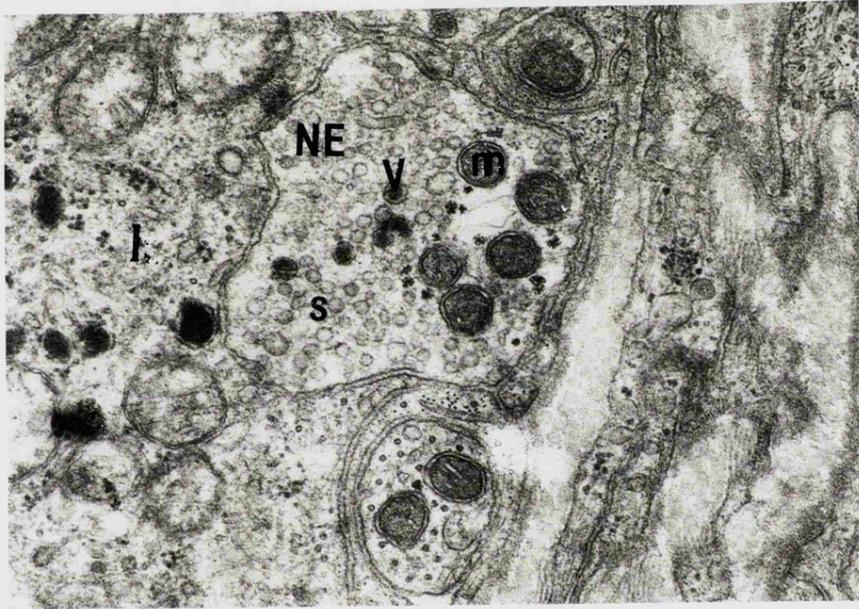


FIGURE 3.15

FIGURE 3.16 A ELECTRON MICROGRAPH SHOWING "AFFERENT" TYPE OF NERVE ENDING - TYPE I CELL SPECIALISED JUNCTION IN CAT CAROTID BODY (x 28,000)

I - Type I cell
NE - Nerve ending
m - mitochondria
S - Synaptic-like vesicles
g - glycogen granules
Arrow - pre-synaptic dense projections

FIGURE 3.16 B ELECTRON MICROGRAPH SHOWING "EFFERENT" TYPE OF NERVE ENDING - TYPE I CELL SPECIALISED JUNCTION IN RAT CAROTID BODY (x 30,000)

I - Type I cell
NE - Nerve ending
S - Synaptic-like vesicles
Arrow - pre-synaptic dense projections

FIGURE 3.16 C ELECTRON MICROGRAPH SHOWING "RECIPROCAL" TYPE OF NERVE ENDING - TYPE I CELL SPECIALISED JUNCTIONS IN CAT CAROTID BODY (x 28,000)

I - Type I cell
NE - Nerve ending
S - Synaptic-like vesicles
v - electron dense-cored vesicle
Arrows - pre-synaptic dense projections

FIGURE 3.16 A

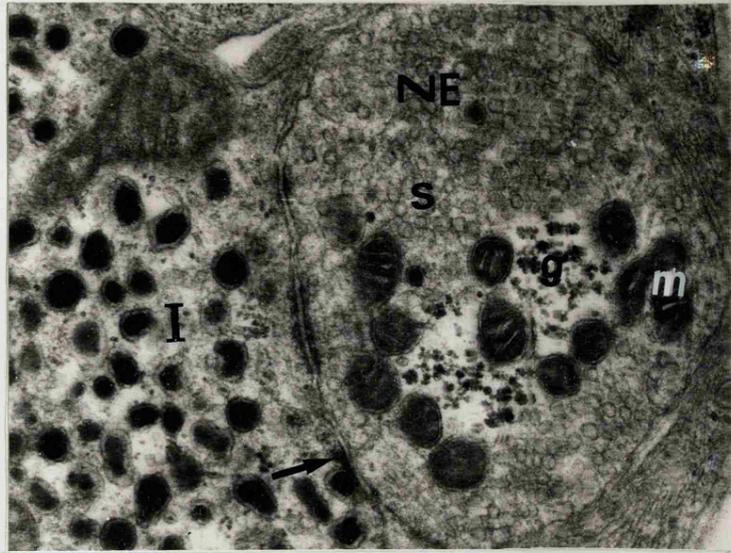
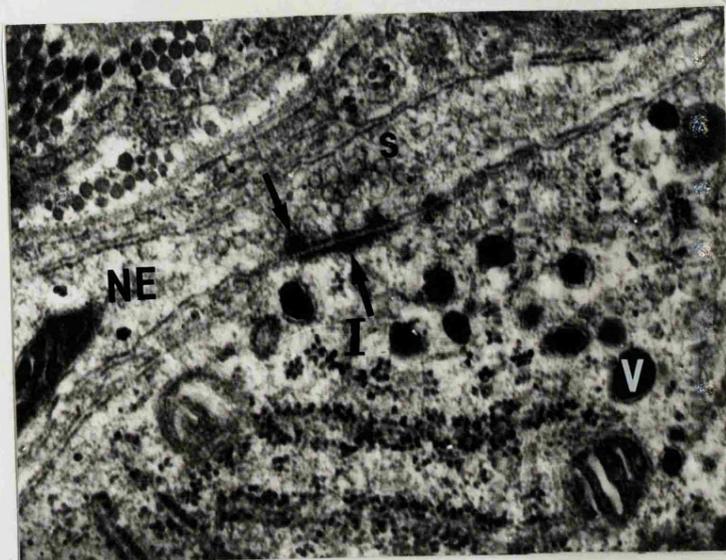


FIGURE 3.16 B



FIGURE 3.16 C



more commonly(c.f. PaLLOT & BLAKEMAN 1982).

3. 3. DISCUSSION

The morphological and general ultrastructural features of cat and rat carotid bodies demonstrated here, are in agreement with the reports of other workers (see Chapter 1).

The close association of Type I cells with the fenestrated capillaries blood vessels observed in all studies of the carotid body, would appear a suitable arrangement whereby a direct route is provided to the Type I cells for any possible $O_2/CO_2/pH$ changes in the blood. This association is particularly marked in areas of Type I cell membrane which lack a covering Type II cell process. The catecholamine studies provide evidence of a direct effect of blood chemistry on the Type I cells (see Chapter 7).

No light or dark varieties of Type I cells, such as those previously described by numerous authors were recognised. Furthermore evidence for a direct release of electron dense-cored vesicles content was never observed in carotid bodies under investigation. Freeze-fracture studies (Hansen, 1977) have, however, shown occasional sites of electron dense-cored vesicle fusion with the plasma membrane of the Type I cell. Gronblad et al (1979), have observed fusion of electron dense cored vesicles with the Type I cell membrane after incubation with calcium transporting ionophore A23137 and 1 mM calcium chloride indicating calcium induced

exocytosis. The lack of evidence of exocytosis of the electron dense-cored vesicles does not mean that it does not occur. The difficulty with observing exocytosis in the Type I cells by the use of normal electron-microscopic techniques is probably a result of rapidity and infrequency of its occurrence.

The differences in the ultrastructure of the cat and rat carotid bodies observed in this study, e.g. the frequencies of contact between Type I cells and occurrences of reciprocal synapses with Type I cells may represent species differences and have effects on the functional behaviour of the carotid bodies of the two types of animals.

The results of stereological analysis of the cat and rat carotid bodies, performed in our laboratory, suggest that there are indeed marked differences between the organs of the two species. For example, in the rat the volume occupied by the unmyelinated nerve fibres is some three times greater than in the cat, and the Type I cell volume density appears much higher.

In both species very nearly 50% of total carotid body volume is composed of non-specific tissue (Ballard et al, 1982; Pallot & Blakeman, unpublished).

Detailed quantitative morphological studies of the carotid bodies in other species may well provide an insight into the different functional behaviour that has been

observed between species and may provide a valuable insight into the mechanism of chemoreception.

CHAPTER FOUR

CHAPTER FOUR

QUANTITATIVE STUDIES OF THE CAT CAROTID BODY TYPE I CELLS

Introduction

The purpose of this study was to provide a quantification of the proportion of the Type I cell cytoplasm occupied by mitochondria and electron dense-cored vesicles. Due to the uncertainty involved in examining tissue removed under varying conditions, animals were therefore ventilated for 30 minutes under normal conditions prior to fixation (see Methods). Two test gases were used - 100% O₂, a condition under which there would be minimal chemoreceptor afferent and efferent activity, and 10% O₂, which causes a marked stimulation of both types of activity.

From these results it was hoped to correlate any structural changes in the tissue with the familiar physiological effects of hypoxia. In the second part of the study the question of the existence of sub-populations of Type I cells based on differences in the size and/or density of electron dense cored vesicles is examined.

4.1. STEREOLOGICAL INVESTIGATIONS

4.1.1. STEREOLOGY OF TYPE I CELLS AFTER VENTILATION WITH 100% O₂

The results obtained from a study of three carotid bodies are illustrated in Table 4.1 and the state of the innervation of the organ during the test gas ventilation period is also shown.

From this data it is clear that the condition of the innervation during ventilation with 100% O₂ has no effect on the Vv% of either the nucleus or E.D.C.V. within the Type I cells. There is, however, an apparent difference in the percentage of cytoplasm occupied by mitochondria (0.05 > p > 0.02, Student's 't' test). It is difficult, however, to decide upon the importance of this apparently significant difference in view of the extreme variability of the individual values for mitochondrial Vv% (see below).

The great variability in the Vv% of both mitochondria and E.D.C.V. is illustrated in Figures 4.1 and 4.2 which show distribution histograms of these parameters for block number 19. Note that whilst the distributions are not normal in either case, it is difficult to distinguish more than one population of cells on the basis of these criteria. Figure 4.1 also illustrates the difficulty in assessing the validity of the apparent differences in mitochondrial Vv% between blocks 19 and 20.

4.1.2. STEREOLOGY OF TYPE I CELLS AFTER VENTILATION WITH 10% O₂ IN N₂

The results obtained from the study of four carotid bodies obtained from three animals are shown in Table 4.2.

BLOCK NUMBER	19	20	22	OVERALL
State of Nerve	Intact	Cut	Intact	
Nuclear Vv%	42.1 ± 13.8	40.7 ± 11.9	39.1 ± 15.4	40.6 ± 13.7
Mitochondrial Vv%	7.1 ± 3.3	8.5 ± 2.7	7.0 ± 3.1	7.5 ± 3.0
p Values	0.05 > p > 0.02			
E.D.C.V. Vv%	12.5 ± 4.5	13.9 ± 4.5	14.3 ± 4.6	13.6 ± 4.5
p Value	0.5 > p > 0.1			
Number of Cells Analysed	50	50	49	149

Table 4.1

Vv% of the nuclei, mitochondria and electron dense cored vesicles after ventilation with 100% O₂.

All values are: mean ± standard deviation.

p value: Student's 't' test.

Blocks 19 and 20 were obtained from opposite sides of the same animal.

FIGURE 4.1 THE DISTRIBUTIONS OF THE VOLUME/VOLUME DENSITIES OF THE MITOCHONDRIA OF TYPE I CELLS FROM CAROTID BODY OF CAT VENTILATED WITH 100% O₂ FOR 30 MINUTES (HCB 19)

FIGURE 4.2 THE DISTRIBUTION OF THE VOLUME/VOLUME DENSITIES OF THE ELECTRON DENSE-CORED VESICLES OF TYPE I CELLS FROM CAROTID BODY OF CAT VENTILATED WITH 100% O₂ FOR 30 MINUTES (HCB 19)

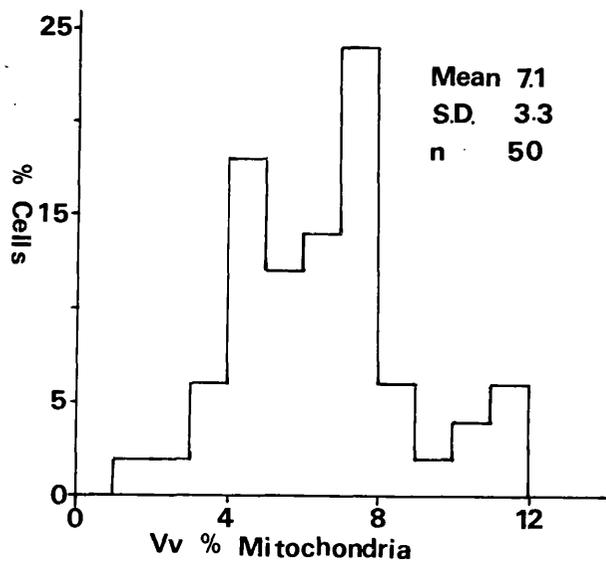


FIGURE 4.1

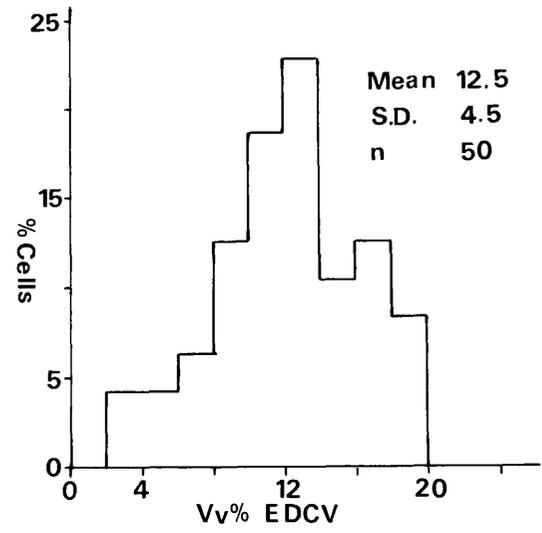


FIGURE 4.2

BLOCK NUMBER	5	6	41	46	OVERALL
State of Innervation	CUT	INTACT	INTACT	INTACT	
Nuclear Vv%	42.3 ± 13.9	38.5 ± 16.3	35.6 ± 14.7	39.3 ± 12.3	38.8 ± 14.3
Mitochondrial Vv%	8.9 ± 2.7	8.0 ± 3.9	9.8 ± 2.7	8.5 ± 2.5	8.9 ± 2.9
p Values	p > 0,5				
E.D.C.V. Vv%	11.1 ± 2.8	10.9 ± 3.0	11.9 ± 3.3	11.7 ± 2.7	11.4 ± 3.0
p Values	p > 0.5				
Number of Cells Analysed	25	25	30	25	105

Table 4.2

Vv% of nuclei, mitochondria and E.D.C.V. after ventilation with 10% O₂ for 30 minutes.

All values are mean ± standard deviation.

Blocks 5 and 6 are from opposite sides of the same animal.

p Value: Student's 't' test.

These illustrate the fact that the state of innervation does not influence the mean value of any of the parameters. The values recorded for individual cells were extremely variable as shown by Figures 4.3 and 4.4 which represent the distribution histograms of mitochondrial and electron dense cored vesicle volume density respectively. The histograms show an approximately normal distribution with no evidence of more than one population of cells.

4.1.3. COMPARISON OF MITOCHONDRIAL AND EDCV Vv% AFTER VENTILATION WITH 10% AND 100% O₂

The pooled data from animals ventilated with 10% O₂ in N₂ and 100% O₂ is illustrated in Table 4.3 from which it is clear that hypoxia does not alter the nuclear volume density. However, there is a significant increase in mitochondrial volume density ($p < 0.001$, Student's 't' test) and it was shown that hypoxia does cause a considerable fall in the proportion of Type I cell cytoplasm occupied by the electron dense cored vesicles ($p < 0.001$, Student's 't' test) (see Figures 4.5 (a) & (b); 4.6 (a) & (b)).

4.2. MORPHOMETRIC INVESTIGATIONS

4.2.1. MORPHOMETRY OF THE TYPE I CELLS OF THE CAT CAROTID BODY

In this study five organs from three animals ventilated with 100% O₂ were used, three of the organs studied were

FIGURE 4.3 THE DISTRIBUTION OF THE VOLUME/VOLUME DENSITIES OF THE MITOCHONDRIA OF TYPE I CELLS FROM CAROTID BODY OF CAT VENTILATED WITH 10% O₂ in N₂ FOR 30 MINUTES (HCB 6)

FIGURE 4.4 THE DISTRIBUTION OF THE ELECTRON DENSE-CORED VESICLES VOLUME (VOLUME DENSITIES OF TYPE I CELLS FROM CAT CAROTID BODY OF CAT VENTILATED WITH 10% O₂ in N₂ FOR 30 MINUTES (HCB 6)

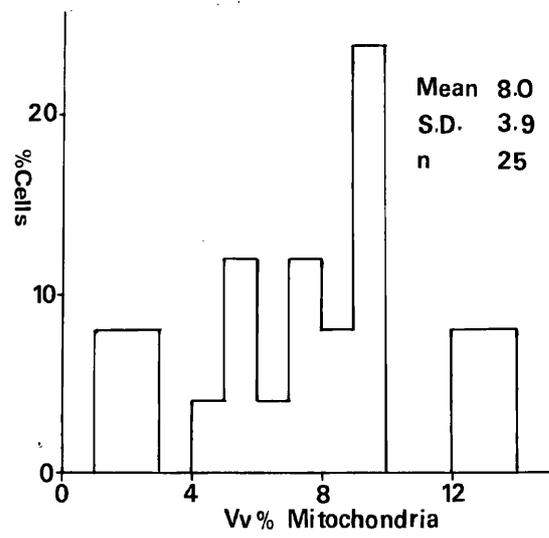


FIGURE 4.3

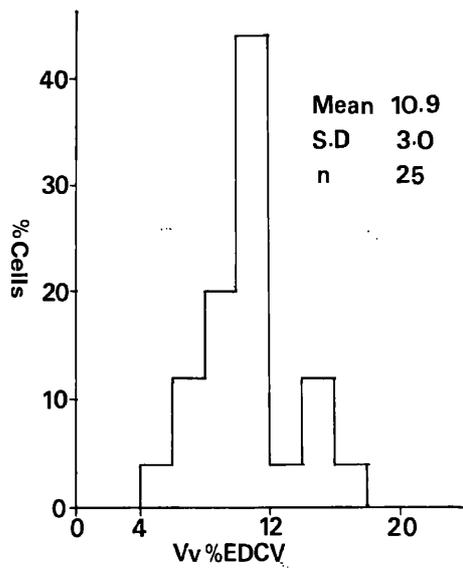


FIGURE 4.4

	ALL BLOCKS n = 254	10% O ₂ IN N ₂ n = 105	100% O ₂ n = 149	p VALUES
Nuclear Vv%	39.9 ± 13.95	38.8 ± 14.3	40.6 ± 13.7	0.5>p>0.1
Mitochondria Vv%	/	8.9 ± 2.9	7.5 ± 3.0	p < 0.001
E.D.C.V. Vv%	/	11.4 ± 3.0	13.6 ± 4.5	p < 0.001

Table 4.3

Pooled data for all Type I cells studied for stereology.

p values for student's 't' test comparing animal ventilated with 10% O₂ in N₂ with 100% O₂.

All values = mean ± standard deviation.

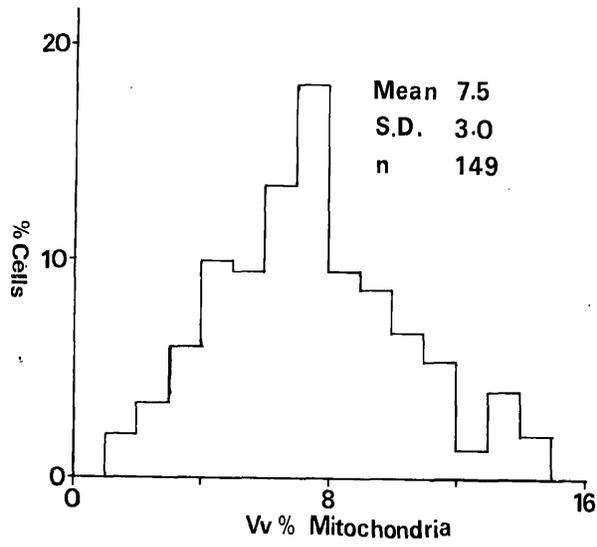
n = number of cells analysed.

FIGURE 4.5 (A AND B) THE DISTRIBUTIONS OF THE MITOCHONDRIAL
VOLUME/VOLUME DENSITIES OF ALL CAT TYPE I
CELLS STUDIED AFTER BEING VENTILATED WITH:

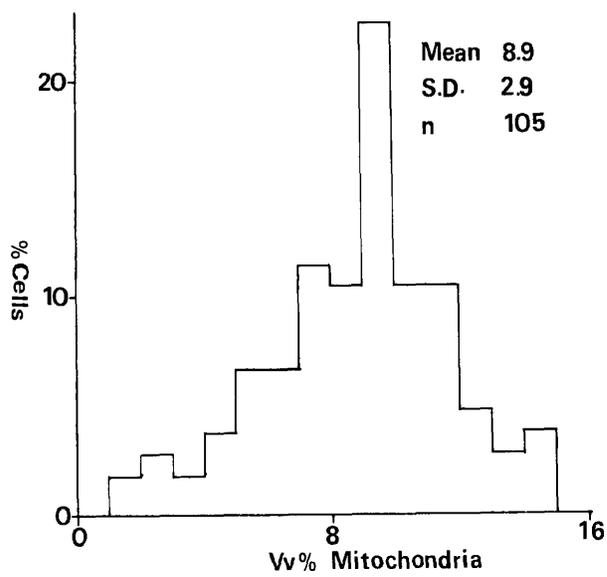
A - 100% O₂

B - 10% O₂ in N₂

FIGURE 4.5



A -



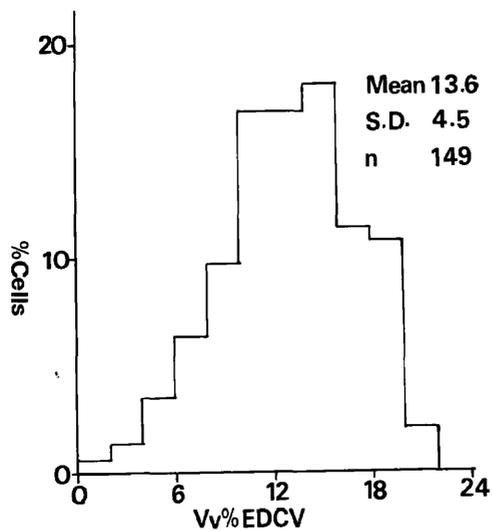
B -

FIGURE 4.6 (A AND B) THE DISTRIBUTIONS OF ELECTRON DENSE-CORED
VESICLE VOLUME/VOLUME DENSITIES OF ALL CAT
TYPE I CELLS STUDIED AFTER VENTILATION FOR
30 MINUTES WITH:

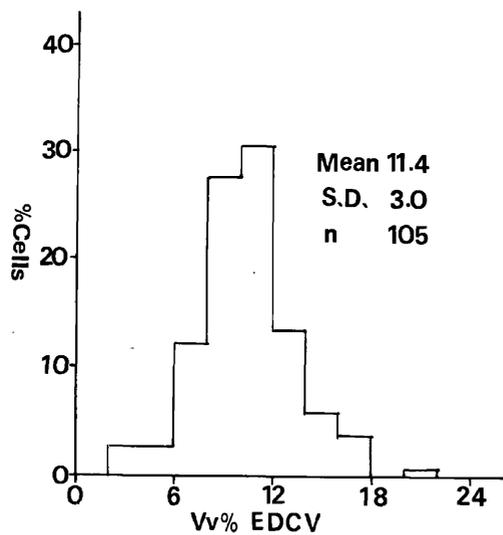
A - 100% O₂

B - 10% O₂ in N₂

FIGURE 4.6



A -



B -

connected to a sinus nerve which had been sectioned prior to ventilation, the other two being connected to an intact sinus nerve.

Table 4.4 shows the area of the nuclei and cytoplasm (mean +/- S.D.) for all of the cells studied. Whilst there are minor variations between animals and individual organs there are no significant differences in the measured cells from any organ.

4.2.2. DETAILED ANALYSIS OF ELECTRON DENSE CORED VESICLES IN TYPE I CELLS

The cells referred to above were used for the study of the diameter and density of the electron dense cored vesicles (see Table 4.5).

A. Electron Dense Cored Vesicle Diameter

The individual diameters of EDCVs were determined for only those vesicles with clearly defined membranes. This usually constituted some 70 - 80% of the total population (average value, total electron dense cored vesicles/cell profile = 196, EDCVs measured = 152.49/cell profile).

Within any cell the range of diameters of individual vesicles was wide, as evidenced by the high standard deviation of the mean values for all vesicles within a single organ (see Table 4.5). It is, therefore, surprising that the median values of vesicle diameter are almost identical to the means. The range of

BLOCK NUMBER	HCB 20	HCB 21	HCB 22	HCB 23	HCB 24	ALL BLOCKS
NUMBER OF CELLS	78	51	85	60	59	333
STATE OF INNervation (C.S.N.)	C.S.N. CUT	C.S.N. CUT	C.S.N. INTACT	C.S.N. INTACT	C.S.N. CUT	
AREA OF CELL	52.50 ± 10.52	59.84 ± 19.06	60.74 ± 19.42	59.93 ± 16.42	62.17 ± 18.28	58.8 ± 16.53
AREA OF NUCLEUS	21.60 ± 7.13	21.69 ± 9.03	22.43 ± 10.03	23.05 ± 8.19	24.02 ± 7.80	22.5 ± 8.5
AREA OF CYTOPLASM	30.93 ± 9.76	38.20 ± 16.15	38.31 ± 16.57	38.86 ± 11.47	38.13 ± 16.27	36.3 ± 13.9

Table 4.4

Showing area of cell, nucleus, and cytoplasm of cat carotid body Type I cell.
Values given as: mean ± S.D. μm^2 .

BLOCK NUMBER	HCB 20	HCB 21	HCB 22	HCB 23	HCB 24	ALL BLOCKS
NUMBER OF CELLS	78	51	85	60	59	333
STATE OF INNervation (C.S.N.)	C.S.N. CUT	C.S.N. CUT	C.S.N. INTACT	C.S.N. INTACT	C.S.N. CUT	
MEAN VESICLE DIAMETER (nm)	148.32 ± 23.34	149.26 ± 23.74	145.07 ± 28.37	146.68 ± 29.09	144.65 ± 29.49	146.69 ± 26.81
MEDIAN OF VESICLE DIAMETER (nm)	147.00 ± 9.67	148.71 ± 8.93	145.67 ± 12.66	148.37 ± 9.97	145.11 ± 9.33	146.83 ± 10.32
NUMBER OF VESICLES MEASURED	11010	7038	14174	9587	8971	50780
EDCV DENSITY (NO./ μm^2 CYTO.)	5.6 ± 1.4	5.2 ± 1.7	5.85 ± 2.0	5.4 ± 1.6	5.3 ± 1.9	5.51 ± 1.72

Table 4.5

Showing Mean Diameter, Median of Diameter and Density of electron dense cored vesicles of cat carotid body Type I cell.

Values given as: mean ± S.D.

vesicle size was from below 8 nm to over 200 nm.

With regard to electron dense cored vesicle diameter in individual cells, these distribution histograms fell into one of three broad categories:

- a) those which had a normal distribution (Figure 4.7a);
- b) those which had a positive skew (Figure 4.7b);
- c) those which appeared to have 2 peaks (Figure 4.7c).

This latter group is small being made up of only some 10 - 15% of the total number of cells analysed; cells of groups (a) and (b) appeared with similar frequency.

An attempt was made to determine whether cell type (c) constituted a separate group with two populations of electron dense cored vesicles. The percentages of the total number of vesicles of each diameter were calculated together with the cumulative percentages for each group (10 nm groups) and these figures were plotted on probability paper (see Figure 4.8). Those cells with one peak yielded a straight line; however, the cells which appeared to contain a sub-population of small vesicles gave a line with two distinct gradients. It would seem likely, therefore, that the peak thus produced does, in fact, reflect the presence of a population of smaller vesicle-containing cells.

The distribution histograms for all the vesicle diameters measured in three carotid bodies from the same number of animals

FIGURE 4.7 (A, B AND C) THE DISTRIBUTIONS OF THE DIAMETERS OF ELECTRON DENSE-CORED VESICLES OF THREE INDIVIDUAL TYPE I CELLS FROM CAROTID BODY OF CAT VENTILATED FOR 30 MINUTES WITH 100% O₂.

THE HISTOGRAMS SHOW CELL WITH:

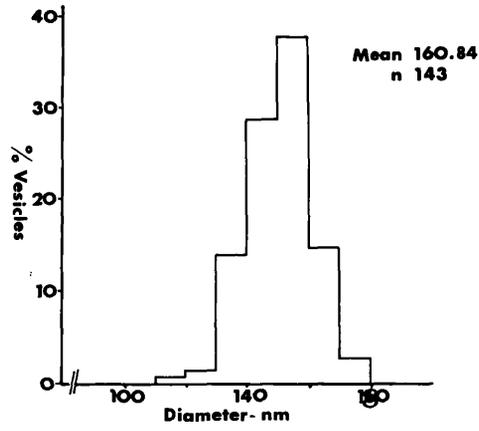
A - NORMAL DISTRIBUTION (ONE PEAK)

B - POSITIVE SKEW

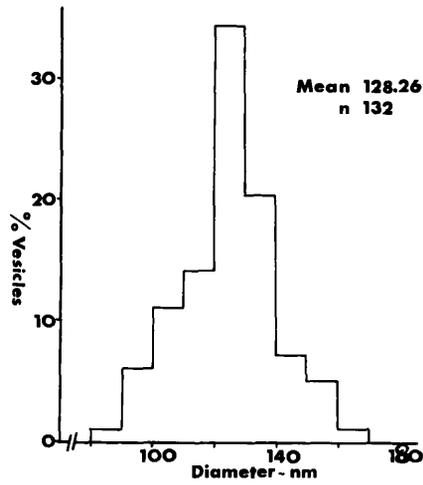
C - TWO PEAKS

FIGURE 4.7

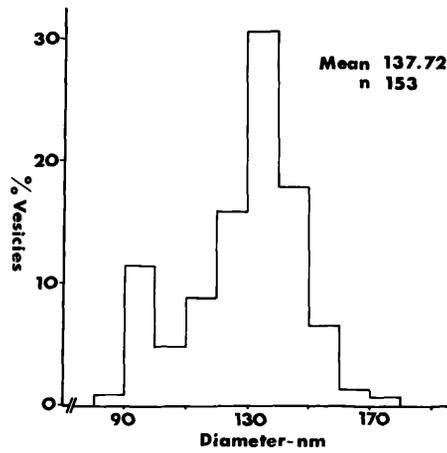
A -



B -



C -



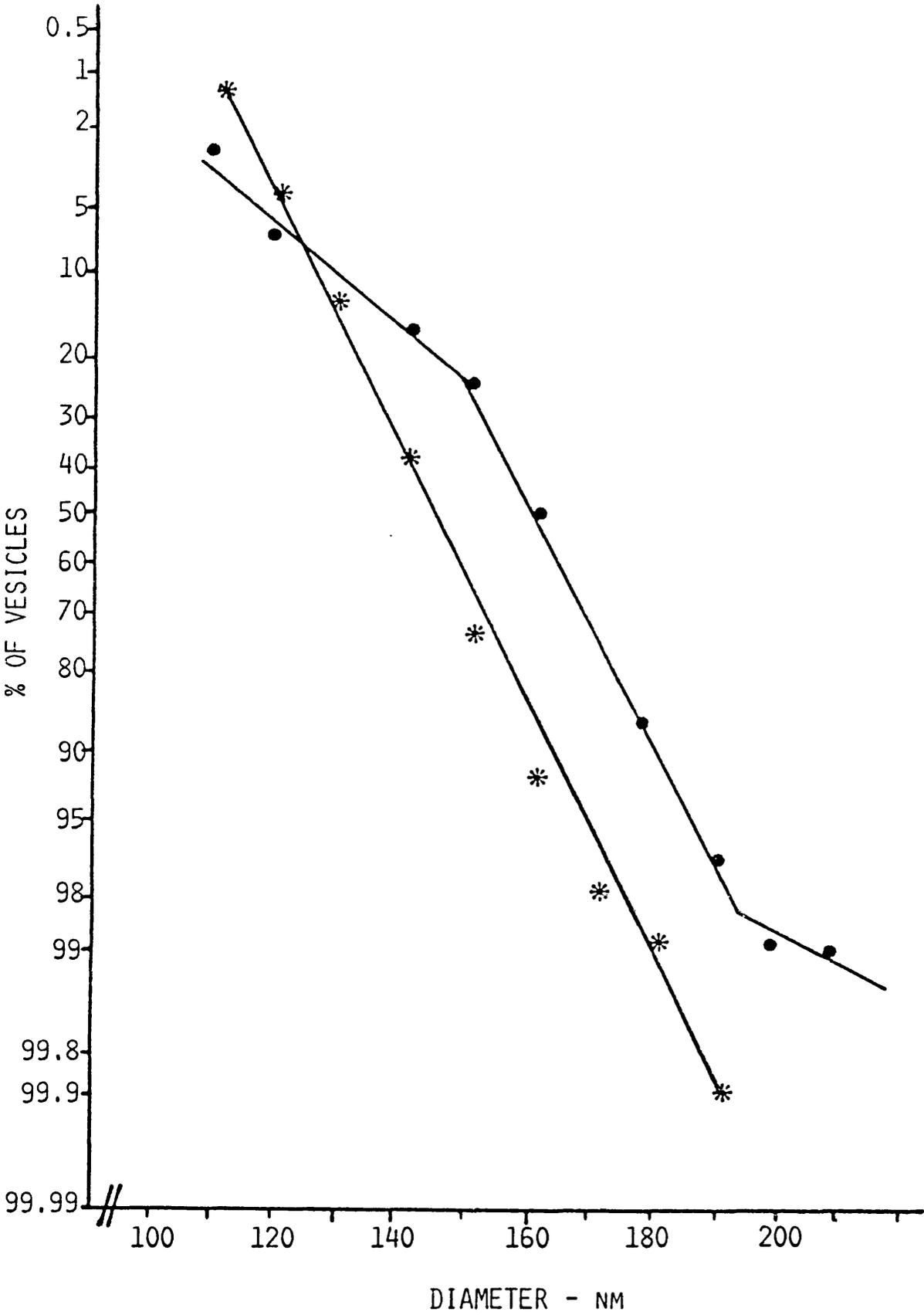


Fig. 4.8: Data taken from four Type I cells showing a normal distribution (*) and four Type I cells showing the bimodal distribution (●)

The percentage of vesicles in each size category is calculated and a cumulative percentage obtained. These values are plotted on probability paper as shown above.

ventilated with 100% O₂ are shown in Figure 4.9 a, b & c. All three blocks show a normal distribution.

The state of innervation did not affect the diameter of the electron dense cored vesicles. The mean values from all the blocks studied were found to be not significantly different in either case (mean values: nerve intact 145.7 +/- 25.4, n = 145; nerve sectioned = 147.4, +/- 28.7, n = 188, p > 0.5, Student's 't' test).

B. The Density of the Electron Dense Cored Vesicles

The density of the EDCVs in each cell was determined. The distribution histograms of the number of vesicles/ μm^2 cytoplasm are shown in Figure 4.10 a, b & c (intact, cut and all blocks respectively). The mean values of the density were slightly lower in the organs where the sinus nerve had been sectioned (mean = 5.4 +/- 1.64) than where the nerve had been left intact (mean = 5.66 +/- 1.83) but they were not significantly different (0.5 > p > 0.1, Student's 't' test). The density of the vesicles was also found to follow a normal distribution. The mean value of electron dense cored vesicle density of all blocks = 5.51 +/- 1.72, n = 333.

The distribution histograms of the EDCV density from intact nerve, cut nerve and all blocks are shown in Figure 4.10 a, b & c respectively.

FIGURE 4.9 (A, B AND C) THE DIAMETER DISTRIBUTION HISTOGRAMS OF ELECTRON DENSE-CORED VESICLES IN ALL TYPE I CELLS FROM THREE INDIVIDUAL CAT CAROTID BODIES VENTILATED WITH 100% O₂ FOR 30 MINUTES.

THE HISTOGRAMS SHOW NORMAL DISTRIBUTIONS.

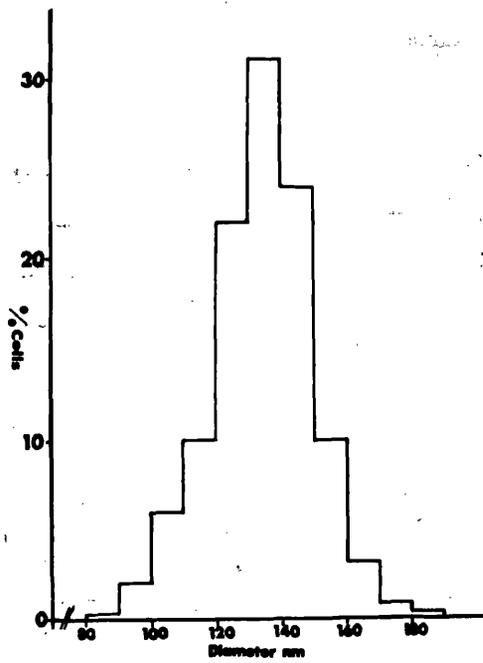
EDCV DIAMETER: MEAN \pm S.D.

A - 149.26 \pm 23.74 (N = 7038, C.S.N. cut)

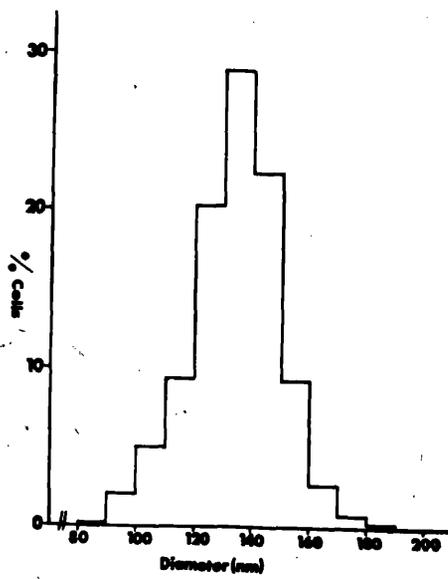
B - 146.68 \pm 29.09 (N = 9587, C.S.N. intact)

C - 144.65 \pm 29.49 (N = 8971, C.S.N. cut)

N = Number of vesicles measured

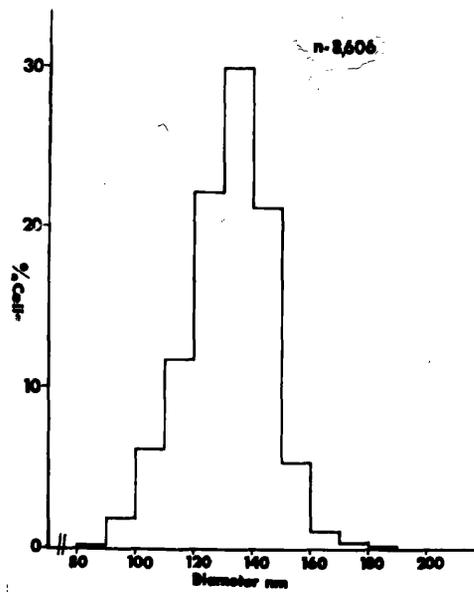


A -



B -

FIGURE 4.9



C -

FIGURE 4.10 (A, B AND C) THE DISTRIBUTION OF DENSITIES
(NUMBER/UM² CYTOPLASM) OF ELECTRON
DENSE-CORED VESICLES IN CAT CAROTID
BODY TYPE I CELLS WITH:

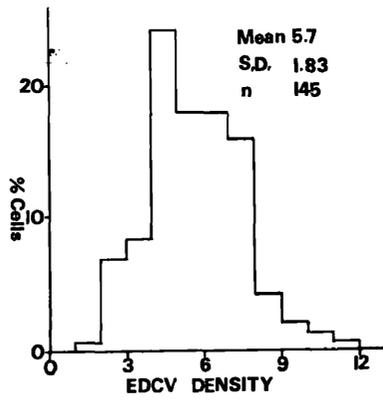
A. Intact Carotid Sinus Nerve.

B. Sectioned Carotid Sinus Nerve.

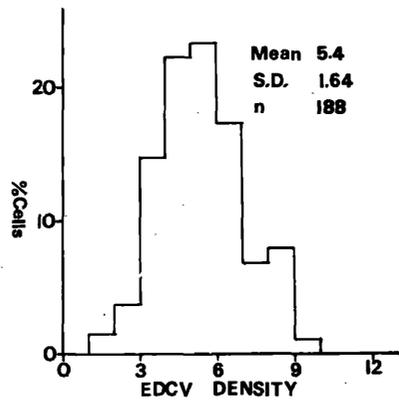
C. All Blocks (intact and sectioned C.S.N.)

FIGURE 4.10

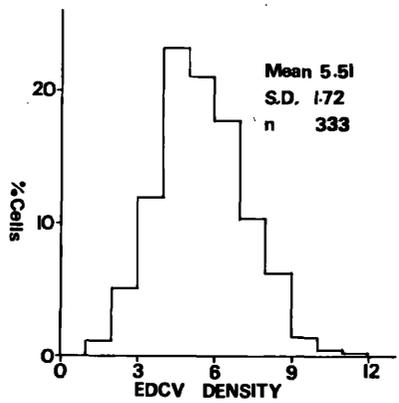
A -



B -



C -



4.3. DISCUSSION

These experiments were conducted in order to examine two main points:

- a) does hypoxic stimulation cause any identifiable structural changes within the cat carotid body?

- b) can more than one variety of Type I cell be identified on the basis of size and EDCV density?

In addition, it was hoped to provide structural data for the catecholamine studies reported below.

With regard to the first question, it may be that the use of 100% oxygen as the baseline condition is criticised as being unphysiological. However, it was felt necessary to provide an adequate controllable baseline for the study and, given that some method had to be employed whereby this could be achieved, ventilation with 100% O₂ ensures minimal chemoreceptor stimulation during the pre-fixation period. 10% O₂ in N₂ was chosen as the 'test gas' since it provides a reasonable chemoreceptor stimulant (see Biscoe et al., 1970) and it also avoids the ludicrously unphysiological conditions of some previous studies (see Chapter 1).

This study has shown that hypoxia does affect the Type I cell stereology of the cat carotid body by reducing the proportion of cytoplasm occupied by electron dense cored vesicles, at the same time considerably increasing the

mitochondrial volume density. It is difficult to compare these results with other reports on the same subject (e.g. Al-Lami & Murray, 1968a) since the latter are qualitative observations rather than systematic, controlled quantitative results.

The increase found in mitochondrial volume density of Type I cells in hypoxia accords with other reports (Al-Lami & Murray, 1968a - cat; Hellstrom, 1977a - rat) indicating swelling of mitochondria and could be due ^{to} increased activity within the Type I cells (c/f McDonald and Mitchell, 1975a). Poor tissue preservation can be excluded.

The reduction in the proportion of Type I cell cytoplasm occupied by EDCVs in hypoxic cats could be due to release of these vesicles from the Type I cells or to vesicle disintegration. The first of these possibilities is thought to be the more likely since these vesicles are reported by many authors to be the catecholamine storage site in the Type I cells (see Chapter 5). In further support of this possibility are the findings of Mills and Slotkin (1975) that, in the cat, hypoxia causes a reduction in the carotid body catecholamine (noradrenaline and adrenaline) content. Similar results in the rat were reported by Hellstrom, Hanbauer and Costa (1976) and Hellstrom (1977a). Regrettably neither the density nor diameter of EDCVs were measured after 10% O₂ and hence the possibility of changes in vesicle size cannot be ruled out.

The finding that the state of the sinus nerve does not affect either the mitochondrial or EDCVs volume densities in Type

I cells suggests that hypoxic stimulation acts locally on the Type I cell rather than centrally via an efferent pathway.

The finding is of some interest for, according to Mills and Slotkin (1975) the release of catecholamines from the cat carotid body during hypoxia is at least partially dependent upon an intact sinus nerve. There is also the question of the function of the efferent pathway described by Morgan et al. (1981) which, in view of the present results, appears redundant.

Our morphometric studies of the diameter and density of the Type I cell electron dense-cored vesicles were an attempt to examine whether or not the Type I cell sub-classes reported by other authors (e.g. in cat - Morita et al., 1969; in the rat - Hellstrom, 1975a; McDonald & Mitchell, 1975a), really exist in the Type I cells of the cat carotid body. There is good evidence presented here that some 10-15% of the total number of cells analysed contain 2 peaks within EDCV diameter histograms - the difficulty is in assessing whether these vesicles constitute a sub-population. According to Verna (1977), only one kind of Type I cells exists in rabbit carotid bodies. He, too, however refers to some cells with bimodal distribution histograms and raises the possibility that these bimodal cells represent cells in different physiological states.

The fact that when all of the values of vesicle diameter for each cell studied in a given organ are combined a unimodal normal distribution is produced, argues that on the criteria of vesicle diameter, only one population of Type I cell exists.

Since the first ultrastructural studies of Type I cells (e.g. Lever & Boyd, 1957) 'light' and 'dark' cells have been described. There is a major difficulty in this sub-division for it is well known that such apparent differences in electron density are often artefact (see Verna, 1979).

Morita et al. (1969) provided the first 'quantitative' data on cat carotid body Type I cells. Noting the presence of light and dark cells in immersion-fixed material these authors sub-divided the dark Type I cells into three types based on the size and density of the EDCV. The total lack of methodological information, so important in such studies, makes interpretation of the validity of this data impossible.

The quantitative data related to rat carotid body EDCV will be discussed. in Chapter 5.

The density of EDCVs in Type I cells, were distributed about a mean of 5.5 vesicles/ μm^2 ; it was not possible to establish the presence of more than one kind of Type I cell in the cat on the basis of this parameter.

In conclusion these studies have shown that there are changes in the Vv densities of mitochondria and EDCV following ventilation with 10% O_2 . The change in EDCV Vv% is in keeping with the reported decrease in catecholamine concentration after 10% O_2 (Mills & Slotkin, 1975). The fact that the changes occur in Type I cells regardless of the state of the innervation rule

out the involvement of any efferent innervation to the organ (see Pallot, 1983; McQueen & Pallot, 1983). Finally, on the basis of EDCV size and diameter we are unable to firmly establish the presence of more than one Type I cell variety. The presence of bimodality in some sized distribution histograms suggests at best that some Type I cells may contain two types of vesicle. Whether these two types of vesicle contain ^{different} compounds or whether they represent different physiological states of the same vesicle type is not established.

CHAPTER FIVE

CHAPTER FIVE

QUANTITATIVE STUDIES OF THE RAT CAROTID BODY TYPE I CELLS

Introduction

The purpose of this investigation, like that carried out on the cat carotid body (Chapter 4), was to quantify the proportion of the Type I cells and cytoplasm occupied by mitochondria and electron dense cored vesicles. There have been many morphological, physiological and biochemical studies performed on the carotid body which suggests the presence of species difference.

In this thesis an attempt has been made to observe the effect of hypoxia and the state of innervation on the rat Type I cells and their profiles and investigate the possible existence of sub-populations of Type I cells on the basis of differences in the size and/or density of EDCV.

The stereological work on Type I cells following 10% oxygen was performed jointly with Miss N. Blakeman.

5. 1. STEREOLOGICAL INVESTIGATIONS

5. 1. 1. STEREOLOGY OF RAT CAROTID BODY TYPE I CELLS FOLLOWING VENTILATION WITH 100% AND 10% O₂ IN N₂-

In this study four carotid bodies taken from the same

number of animals ventilated with 100% O₂ for 30 minutes, and three taken from two animals ventilated with 10% O₂ in N₂ were used.

Table 5.1 shows the results from carotid bodies ventilated with 100% O₂ and Table 5.2 shows the results after ventilation with 10% O₂ in N₂.

The mean values for both groups of data are less variable than those found in the cat. The mitochondrial volume/volume ratio within a particular group are relatively constant, as shown in Figures 5.1A & B. and Table 5.1 and do not appear to be affected by the state of innervation. Thus, in one rat (number 9), both carotid bodies were removed following ventilation with 10% O₂ in N₂ for 30 minutes, the right hand carotid sinus nerve being sectioned prior to the period of hypoxia. The mitochondrial volume/volume ratio was similar following sinus nerve section when compared to that in the control carotid body taken from the left hand side ($0.1 > p > 0.05$, Student's 't' test).

The state of innervation does not change to any considerable extent the volume/volume ratio of electron dense cored vesicles ($0.5 > p > 0.1$, Student's 't' test). Although there is a wide range of values both within and between animals, conclusive evidence has not been found to suggest that there is more than one distinct population of vesicles based on this parameter.

	ANIMAL			
	1	2	3	4
n	31	38	30	32
Nuclear Vv%	40.08 ± 11.36	47.66 ± 13.22	41.04 ± 15.85	48.26 ± 11.9
Mitochondrial Vv%	10.33 ± 1.99	10.24 ± 3.80	10.03 ± 3.88	9.32 ± 2.69
EDCV Vv%	2.26 ± 1.04	2.45 ± 1.19	2.63 ± 0.92	2.40 ± 1.15

Table 5.1

Volume/volume ratio expressed as Vv% for Type I cells from animals ventilated with 100% O₂.

Values given as $\bar{x} \pm S.D.$

n = Number of Type I cells analysed.

	ANIMAL		
	8 (RHS)	9 (LHS)	9 (RHS)
n	30	31	32
Nuclear Vv%	46.72 ± 10.57	41.44 ± 13.65	43.01 ± 13.58
Mitochondrial Vv%	9.09 ± 1.98	10.83 ± 5.12	13.36 ± 5.13
'p' value		0.1 > p > 0.05	
EDCV Vv%	4.02 ± 1.71	4.88 ± 1.96	4.29 ± 1.87
'p' value		0.5 > p > 0.1	

Table 5.2

Volume/volume ratio expressed as Vv% for Type I cells from animals ventilated with 10% O₂ in N₂.

Values given as x ± S.D.

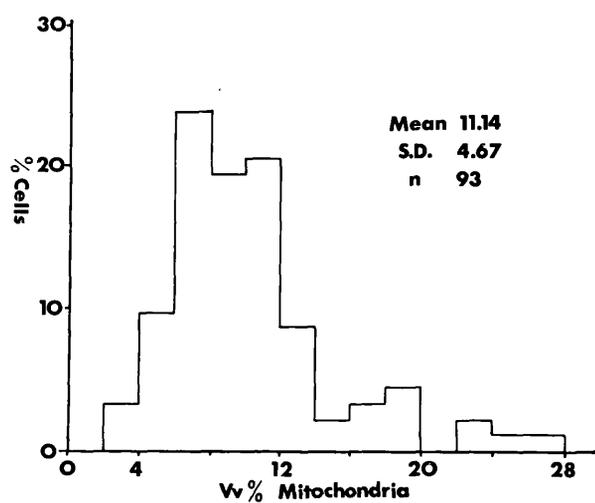
'p' values derived using student's 't' test.

FIGURE 5.1 (A AND B) THE DISTRIBUTIONS OF VOLUME/VOLUME
DENSITY OF THE MITOCHONDRIA OF TYPE I
CELLS FROM THE CAROTID BODIES OF
RATS VENTILATED FOR 30 MINUTES WITH:

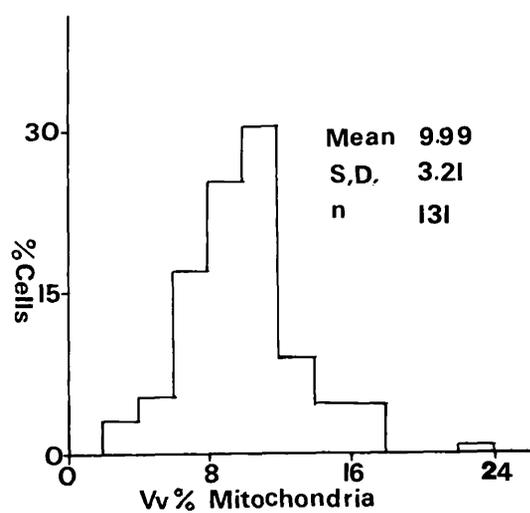
A - 10% O₂ in N₂

B - 100% O₂

FIGURE 5.1



A -



B -

5. 1. 2. COMPARISON OF MITOCHONDRIAL AND EDCV Vv% AFTER VENTILATION WITH 10% O₂ IN N₂ AND 100% O₂

The pooled data from all blocks used in these investigations is shown in Table 5.3. The mitochondrial volume/volume ratio appears to be affected by the gas used for ventilation (or by hypoxia); the overall mean following hypoxia is significantly higher than that following hyperoxia ($0.05 > p > 0.02$, Student's 't' test). The distribution histograms for these ratios from animals ventilated with 10% O₂ in N₂ and 100% O₂ are shown in Figure 5.1 (a) and (b) respectively.

Hypoxia also appears to affect the volume occupied by the electron dense cored vesicles - the volume/volume ratio of EDCVs is much greater in carotid bodies subjected to the hypoxic stimulus than compared with those ventilated with 100% O₂ ($p > 0.001$, Student's 't' test). The distribution histograms for those subjected to 10% O₂ in N₂ and 100% O₂ are shown in Figure 5.2 (a) and (b) respectively.

5. 2. MORPHOMETRIC INVESTIGATIONS OF THE ELECTRON DENSE CORED VESICLES : THE DIAMETER AND DENSITY

5. 2. 1. THE DIAMETER OF THE ELECTRON DENSE CORED VESICLES

In this study four carotid bodies from four animals ventilated with 100% O₂ for 30 minutes were used. The

	10% O ₂ in N ₂ n = 93	100% O ₂ n = 131	'p' Values
Nuclear Vv%	43.69 ± 12.82	44.49 ± 13.52	
Mitochondrial Vv%	11.14 ± 4.67	9.99 ± 3.21	0.05 > p > 0.02
EDCV Vv%	4.37 ± 1.82	2.43 ± 1.08	p > 0.001

Table 5.3

Pooled data from all blocks used in this study.

Value given as $\bar{x} \pm \text{S.D.}$

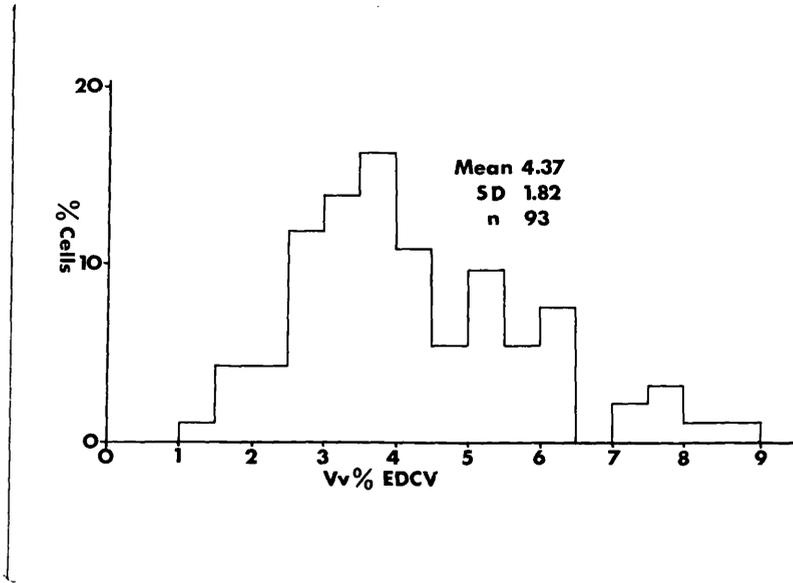
'p' value derived using student's 't' test.

FIGURE 5.2 (A AND B) THE DISTRIBUTIONS OF THE VOLUME/VOLUME
DENSITIES OF ELECTRON DENSE-CORED VESICLES
FROM TYPE I CELLS OF THE RAT CAROTID
BODY AFTER VENTILATION FOR 30 MINUTES
WITH:

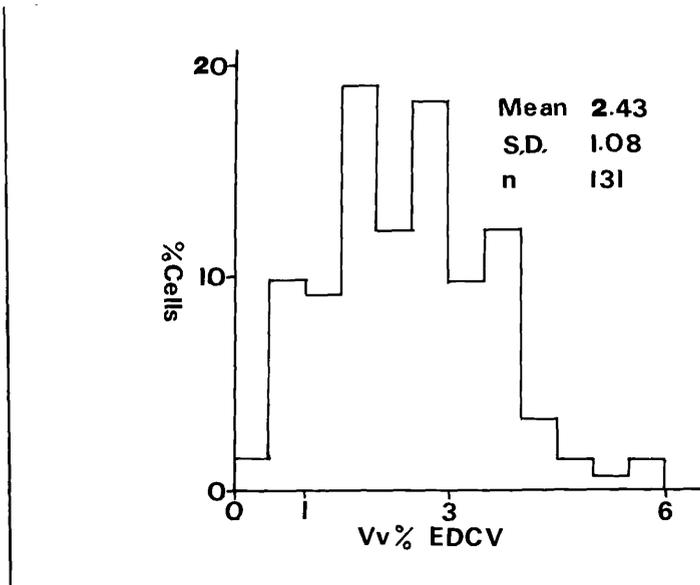
A - 10% O₂ in N₂

B - 100% O₂

FIGURE 5.2



A -



B -

diameters of all the Type I cell EDCVs possessing a clear outer membrane were measured; vesicles satisfying these criteria constituted about 60% of the total cell population.

Table 5.4 shows the individual mean values of EDCV diameters for each carotid body; their distribution histograms are shown in Figure 5.3 (a) to (d).

All the carotid bodies have normal distributions with a mean EDCV diameter of about 100nm; evidence has not yet been found to suggest that there is more than one population of cells. There were a wide range of vesicle diameters within individual cells, (e.g. 40 nm to 135 nm, 104 vesicles measured in one cell). The overall mean of each carotid body is not significantly different from each other ($p < 0.5$, Student's 't' test in all cases); the distribution histogram for all the values is shown in Figure 5.4 (overall mean = 100.69 ± 17.12 nm S.D.; $n = 9\ 903$ vesicles from 161 cells). An average of 61 vesicles/cell was measured (range 12 to 185).

5. 2. 2. THE DENSITY OF ELECTRON DENSE CORED VESICLES

The Type I cells average cytoplasmic area in the rat was not affected by hypoxia or hyperoxia - the mean, following 10% O_2 in N_2 , was $34.70 \pm 12.84 \mu m^2$ (mean \pm S.D., $N = 100$) and, following 100% O_2 , was $33.58 \pm 13.12 \mu m^2$ (Mean \pm S.D., $N = 91$). These values are not significantly different ($p < 0.5$, Student's 't' test). The mean cytoplasmic

Animal	Mean Vesicle Diameter (nm)	No. of Vesicles Measured	No. of Cells Used
RAT 1	101.1 ± 16.34	2553	36
RAT 2	100.18 ± 19.21	2362	41
RAT 3	100.53 ± 15.74	2554	44
RAT 4	100.92 ± 17.21	2434	40
ALL BLOCKS	100.69 ± 17.12	9903	161

Table 5.4

The individual mean values of the electron dense cored vesicles for each carotid body.

Values given: Mean ± S.D. (nm).

FIGURE 5.3 (A, B, C AND D) THE DISTRIBUTION OF THE DIAMETERS OF ELECTRON DENSE-CORED VESICLES FROM RAT TYPE I CELLS. FOUR INDIVIDUAL CAROTID BODIES OBTAINED FROM FOUR DIFFERENT ANIMALS ARE SHOWN.

ALL HISTOGRAMS SHOW APPROXIMATELY NORMAL DISTRIBUTIONS.

EDCV DIAMETER: MEAN \pm S.D. (nm):

A - 101.1 \pm 16.34 (N = 2553)

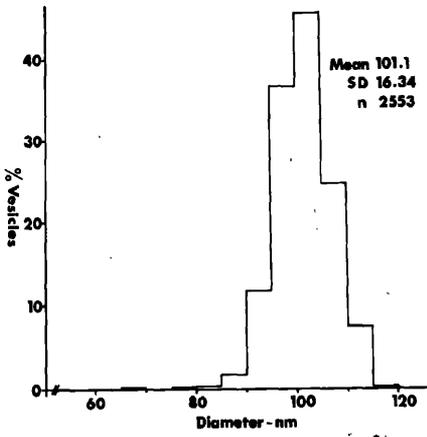
B - 100.18 \pm 19.21 (N = 2362)

C - 100.53 \pm 15.74 (N = 2554)

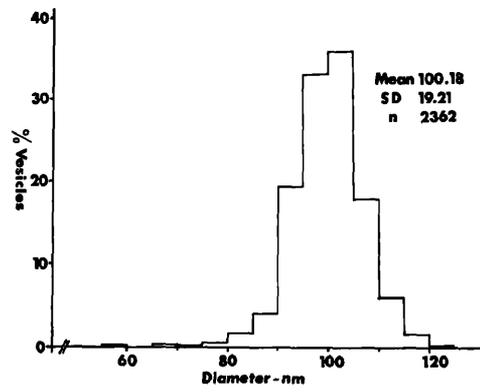
D - 100.92 \pm 17.21 (N = 2434)

N = Number of vesicles measured

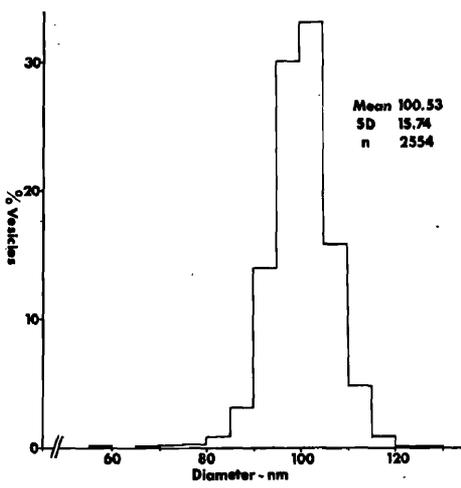
FIGURE 5.3



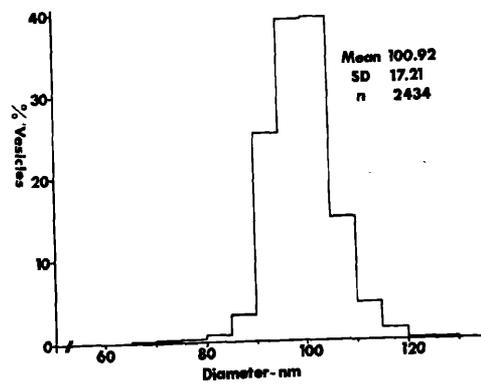
A -



B -



C -



D -

FIGURE 5.4 THE DISTRIBUTIONS OF THE DIAMETER OF ALL ELECTRON
DENSE-CORED VESICLES OF RAT TYPE I CELLS STUDIED.

ELECTRON DENSE-CORED VESICLES MEAN \pm STANDARD
DEVIATION = 100.69 ± 17.12 μm

NUMBER OF VESICLES MEASURED = 9903

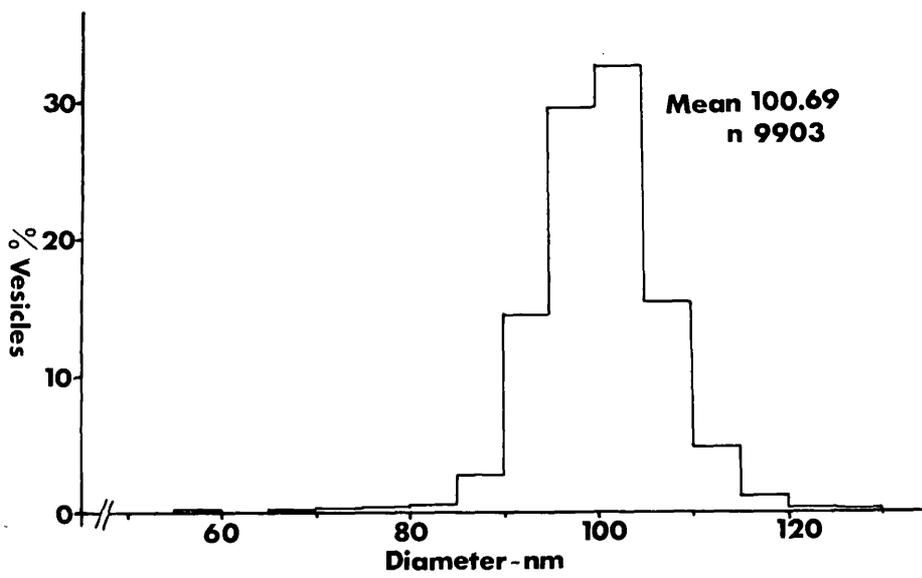


FIGURE 5.4

area of the Type I cells in both hyperoxic and hypoxic rat was $34.12 \pm 12.94 \mu\text{m}^2$ (Mean \pm S.D., N = 191) (see Table 5.5).

There was considerable variation in the mean density of EDCVs between the different carotid bodies, e.g. $2.96 \pm 1.21/\mu\text{m}^2$ in one block compared with $4.27 \pm 1.44/\mu\text{m}^2$ in another, both were from animals ventilated with 100% O_2 (values given as mean \pm S.D., n = 29 and 31 respectively).

The overall mean density of vesicles/ μm^2 for Type I cells taken from carotid bodies subjected to hyperoxia was $3.55 \pm 1.65/\mu\text{m}^2$ (mean \pm S.D., N = 215).

In rats subjected to the hypoxic stimulus of ventilation with 10% O_2 in N_2 for 30 minutes the mean value for EDCV density was decreased to $3.18 \pm 1.14/\mu\text{m}^2$ (Mean \pm S.D., N = 100). This value is significantly lower than that following ventilation with 100% O_2 ($0.05 > p > 0.02$, Student's 't' test).

The distribution histograms for the vesicle densities from carotid bodies subjected to 10% O_2 in N_2 and 100% O_2 are shown in Figure 5.5 (a) and (b). Table 5.6 shows the mean density of vesicles/ μm^2 for individual blocks.

The overall mean value for the number of EDCVs/cell was $116.1 \pm 57.7/\text{cell}$ for hyperoxic blocks and $109.3 \pm 51.4/\text{cell}$ for hypoxic blocks (Mean \pm S.D., N = 91 and 100

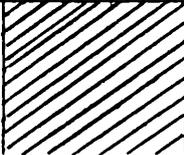
	ALL BLOCKS n =	10% O ₂ IN N ₂ n =	100% O ₂ n =	
Cytoplasmic Area (μm ²)	34.12 ± 12.94 n = 191	34.70 ± 12.84 n = 100	33.58 ± 13.12 n = 91	p < 0.5
EDCV Density (No. of ves./ μm ² cyto.)		3.18 ± 1.14 n = 100	3.55 ± 1.65 n = 215	0.05 > p > 0.02

Table 5.5

Cytoplasmic area and EDCV density.

Value = Mean ± S.D.

n = number of Type I cells used (215 cells were used to determine the EDCV density in hyperoxic rats)

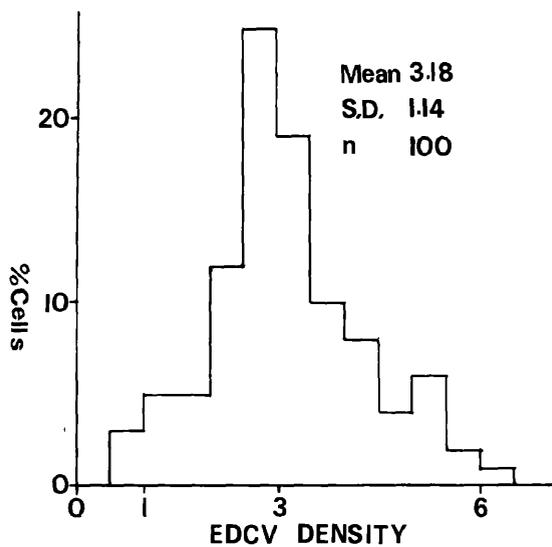
p values Student's 't' test.

FIGURE 5.5 (A AND B) THE DISTRIBUTION OF ELECTRON DENSE-
CORED VESICLE (NUMBER OF EDCVS/ μM^2)
DENSITY IN TYPE I CELLS FROM RAT
CAROTID BODIES VENTILATED FOR 30
MINUTES WITH:

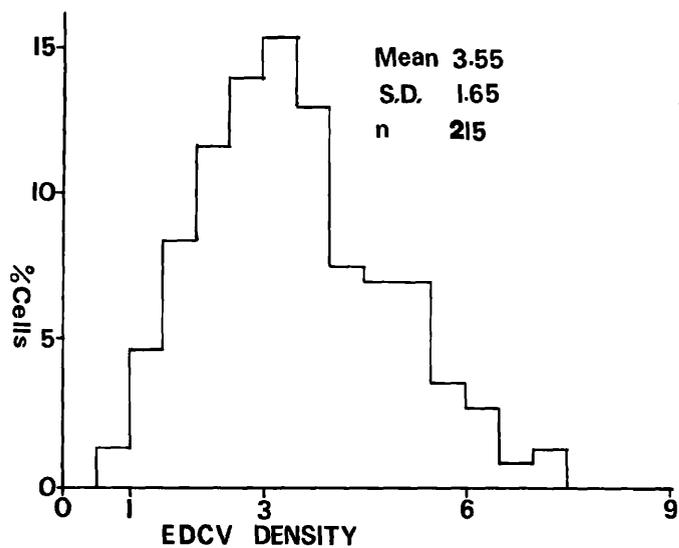
A - 10% O_2 in N_2

B - 100% O_2

FIGURE 5.5



A -



B -

BLOCK	STIMULUS	MEAN VESICLE DENSITY No/ μm^2 CYTOPLASM	n
1	100% O ₂	3.08 \pm 1.27	31
2	100% O ₂	3.36 \pm 1.48	31
3	100% O ₂	4.31 \pm 2.43	30
4	100% O ₂	3.49 \pm 1.49	32
6L	100% O ₂	4.27 \pm 1.44	31
11R	100% O ₂	2.96 \pm 1.21	29
11L	100% O ₂	3.41 \pm 1.58	31
8R	10% O ₂ in N ₂	3.46 \pm 1.20	31
9R	10% O ₂ in N ₂	3.16 \pm 1.15	36
9L	10% O ₂ in N ₂	2.96 \pm 1.05	33

Table 5.6

Mean electron dense cored vesicle densities.
For individual carotid bodies.

Values given: Mean \pm S.D.

n = number of cells analysed

respectively); they are not significantly different ($0.5 > p > 0.1$, Student's 't' test).

The state of innervation did not affect the EDCV density - the mean value for Type I cells from the carotid body with intact innervation (Rat 9 right side) being 3.16 ± 1.15 and 2.96 ± 1.05 for Type I cells from the carotid body in which the carotid sinus nerve had been sectioned (Rat 9 left side) (values given as mean \pm S.D., $N = 36$ and 33 respectively). These values did not differ significantly ($0.5 < p > 0.1$, Student's 't' test).

5. 3. DISCUSSION

Hypoxic stimulation produced quantitative changes in both the electron dense-cored vesicles and mitochondria of the rat carotid body Type I cells.

There is a significant reduction in the density of the electron dense-cored vesicles following hypoxia; a result in agreement with the studies of McDonald and Mitchell (1975a,b) and Hellstrom (1975 a); in 1977 the latter reported this decrease in vesicle numbers in conjunction with a corresponding decrease in the dopamine content as being incrementally proportional to the severity of the hypoxic stimulus.

Although the vesicle density is reduced by hypoxia, the proportion of cytoplasm occupied by the vesicles is

significantly increased and, as there is no change in cells size as a response to the hypoxia, the increase must be due to changes in the vesicle diameter. There are several explanations for this. If hypoxia lead to an increase in catecholamine synthesis in the rat (as has been reported in the cat carotid body - Sampson et al., 1975) this could lead to an expansion in vesicle size. However Hanbauer and Hellstrom (1978) showed that the rate of catecholamine synthesis was constant during hypoxia in the rat. It may be, therefore, that the vesicles take up catecholamines from a cytoplasmic pool leading to a re-distribution of the stores rather than an increase in synthesis. The level of Type I cell catecholamines may be increased in the vesicles prior to their release. Fusion of the electron dense cored vesicles prior to their release is another possibility. Alternatively the increase in volume may be due to the uptake of substances other than catecholamines; the present study cannot isolate one explanation from another. Unfortunately the extent of the increase in the electron dense cored vesicles size in rat Type I cells subjected to hypoxia was not measured in this study.

In this study, the mitochondrial volume/volume ratios of Type I cells from hypoxic rats was significantly higher than those of hyperoxic rats; a result in agreement with the findings of Hellstrom (1977 b), who reported a volume increase following 5% O₂ in N₂. It has been reported that mitochondrial swelling occurs during increased activity (McDonald & Mitchell, 1975a,b).

Activity in the rat sinus nerve does not appear to lead to any significant changes in mitochondrial or electron dense cored vesicle volume/volume ratio during hypoxia, when compared to the control side in which the nerve had been sectioned. Unfortunately other studies upon the affect of the innervation have been completed in cats (Sampson et al., 1975; Mills & Slotkin, 1975) and, in the light of the species differences already described, it does not seem plausible to use their results in the interpretation of these findings. In rat carotid body Hanbauer and Hellstrom (1978) reported that the magnitude of dopamine decrease was similar in intact carotid bodies and in those deprived of carotid sinus nerve innervation. So it seems the hypoxic stimulus has a direct effect on Type I cells. In this respect it is interesting that quantitative studies of the nerve endings in the rat carotid body showed few efferent endings terminating on rat Type I cells(Blakeman & Pallot, unpublished)

Table 5.7 shows the electron dense cored vesicle diameter reported by several authors. Hess (1975) and Morgan et al (1975) reported one population of Type I cells, Gronblad and Eranko (1978) described the diameter of electron dense cored vesicles measured after using different methods of tissue fixation, Hellstrom (1975a) described two populations of Type I cells 'large' and 'small' and McDonald and Mitchell (1975a,b) reported two populations of Type I cells - one cell type (Type A) has 30% larger and more numerous vesicles, than the second (Type B); these two types

AUTHORS	EDCV DIAMETER	SUB-TYPES OF TYPE I CELLS REPORTED
A. Hess (1975)	60-90 nm	One type
M. Morgan et al., 1975	927 ± 185 Å	One type
McDonald and Mitchell, 1975	116 nm 90 nm	TYPE I (A) TYPE I (B)
S. Hellstrom (1975)	71 nm 52 nm	Large Small
* M. Gronbald and O. Eranko (1978)	110-150 nm 90-130 nm 50-120 nm	Fixation with glutaraldehyde. Fixation with neutral permanganate. Fixation with acid permanganate (Rechardt et al., 1977)
This study	100.69 nm	One type

Table 5.7

The EDCV diameter and subtypes of the rat Type I cell reported by different authors and this study.

* Shows the effect of the fixation method on EDCV diameter.

were present in almost equal numbers. These observations were based upon data from a small selection of cells which had been already designated (A) or (B) on the basis of qualitative observations. No reference is made to any attempt at randomising the samples used and making them representative.

In this study, there is no evidence of the presence of more than one population of Type I cells within the rat carotid body, either on the basis of vesicle diameter or density. If we use the method employed by McDonald and Mitchell and designate the cells as large (A) and small (B) without using a random sample technique, the result illustrated in Table 5.8 is obtained. We have cells with an electron dense cored vesicle diameter some 15% larger than type (B) cells. The mean diameters are significantly different ($0.05 > p > 0.02$, Student's 't' test) and the vesicle density is about four times higher in type (A) cells. The results are similar to those described by McDonald and Mitchell (1975a,b).

If, as McDonald and Mitchell claim, the type (A) and (B) cells are present in equal numbers it does not seem unreasonable to expect them to appear with comparable frequency in a random sample such as that used here. When the mean diameters of the two groups are combined an overall mean of 106 nm is obtained; this is close to that described earlier (see Table 5.4) given that fixation and dehydration schedules differed quite markedly.

	TYPE (A) (Large)	TYPE (B) (Small)
EDCV Diameter mean \pm S.D.	105 \pm 15.54 nm	92 \pm 24.22 nm
'p' Value	0.05 > p > 0.02	
Number of Cells Evaluated	23	27
Number of EDCV Measured	2512	908
Average Number EDCV/Cell	109.2	33.6
Average Vesicle Density	3.4	1.10

Table 5.8

Shows EDCV mean diameter and their average number and density per cell from the Type I cells pre-selected as Large Type (A) and Small Type (B) cells.

'p' values: Student's 't' test.

According to this study, and the information available to date, it seems likely that the two cell types previously described represent in reality, the two extremes of a widely distributed population. Indeed McDonald and Mitchell, (1975a,b) stated [“]a transitional type of subtypes of A or B distinguished by our criterion, may exist[”]. These findings serve to emphasise the importance of an unbiased sampling technique. We will return to the question of subtypes of Type I cells in Chapter 6; it is the authors view that the differences in the results reported here and those of McDonald & Mitchell(1975a,b) are purely due to the analysis of a biased population.

CHAPTER SIX

CHAPTER SIX

CATECHOLAMINE CONTENT STUDIES OF THE RAT CAROTID BODY

INTRODUCTION

The catecholamine content of the rat carotid body has been reported by many authors (see Chapter 1). However, the values reported by different authors and, in some cases, by the same authors in different publications are very variable. The question of whether dopamine or noradrenaline is the predominant amine in the rat carotid body required to be reviewed after the report of Mills et al (1978) that, in the cat, noradrenaline rather than dopamine is predominant. This study is an attempt to clarify the conflicting reports and to find the best experimental procedures for establishing the true endogeneous catecholamine levels in the rat carotid body tissue. The carotid bodies were removed from living rats of different strains (including spontaneously hypertensive ones) or from animals killed by different methods in order to examine the effects of anaesthesia and postmortem changes in catecholamine content. Surgical and chemical (6-OHDA) sympathectomy was performed to establish the sympathetic contribution to the rat carotid body.

6.1.1. EFFECT ON THE CAROTID BODY CATECHOLAMINE CONTENT OF DIFFERENT METHODS OF RAT SACRIFICE

Table 6.1. shows the individual values for noradrenaline (NA) and dopamine (DA) in 12 carotid bodies removed from 6

RAT NO. NO. OF CAROTID BODIES	NORADRENALINE	DOPAMINE
R1R	15	12.1
R1L	17.3	11.2
R2R	4.4	9.2
R2L	14.1	7.1
R3R	20.8	7.1
R3L	17	7.9
R4R	7.4	1.8
R4L	15.1	8.2
R5R	14.1	8.2
R5L	28.8	11.2
R6R	8.9	4.1
R6L	17.8	7.6
Mean \pm S.E.	15.06 \pm 1.84	7.98 \pm 0.85

Table 6.1

Showing the noradrenaline and dopamine content in rat carotid bodies removed under anaesthesia from live rats.

Values given as p mol/carotid body.

anaesthetised Sprague-Dawley rats. The mean value for noradrenaline was 15.06 +/- 1.84 p mol (Mean +/- S.E.) and for dopamine; 7.98 +/- 0.85 p mol (mean +/- S.E.); values given as p mol/carotid body.

There is great variability in the values between carotid bodies of different animals, and between the left and right side of the same animal e.g. carotid body R2R is the right side and R2L is the left from the same rat; their noradrenaline levels are 4.4 and 14.1 p mol/carotid body respectively. The variability in all dopamine, while still apparent, is less marked.

The noradrenaline/dopamine ratio (DA/NA) ranges between 1.2 and 4.1.

Table 6.2 shows the individual values for noradrenaline and dopamine, in 12 carotid bodies removed from 6 (S.D.) rats killed by cervical dislocation (C.D.).

The mean values for NA and DA (mean +/- S.E.) are 16.01 +/- 1.31, 6.48 +/- 0.97 p mol/carotid body, respectively. The NA/DA ratios range between 1 and 7.8 with an average of 2.5. Again there is also variability in the NA and DA values of different carotid bodies.

The results of NA and DA determinations in carotid bodies removed from (S.D.) rats after exposure to microwave irradiation (M.W.) are shown in Table 6.3. It may be seen that levels of both amines are much lower when compared to the values in Tables 6.1

NO. OF CAROTID BODY	NORADRENALINE	DOPAMINE	NO. OF GANGLION	NORADRENALINE	DOPAMINE
R1R	14.1	1.8	G1R	150.0	16.5
R1L	16.3	3.0	G1L	174.5	21.7
R2R	22	9.0	G2R	203.8	21.7
R2L	20.4	7.5	G2L	167.9	21.7
R3R	8.4	5.0	G3R	207.5	21.7
R3L	10.8	10.0	G3L	208.5	19.6
R4R	10.0	10.0	G4R	-	-
R4L	19.0	12.7	G4L	188.7	21.7
R5R	14.4	3.7	G5R	168.9	26.1
R5L	18.8	6.3	G5L	205.7	23.9
R6R	16.7	5.0	G6R	181.1	21.7
R6L	21.2	3.8	G6L	186.8	19.6
Mean ± S.E.	16.01 ± 1.31	6.48 ± 0.97	Mean ± S.E.	185.76 ± 5.84	21.45 ± 0.74

Table 6.2

NA and DA content in carotid bodies and S.C.G. of rats killed by cervical dislocation.
 Values given as p mol/carotid body or p mol/S.C.G. (superior cervical ganglion).

M.W.			ANAESTHESIA + M.W.			CERVICAL DISLOCATION + M.W.		
C.B. NO	NA	DA	C.B. NO	NA	DA	C.B. NO	NA	DA
R1R	12.6	0.6	R1R	2.6	1.7	R1R	10.0	1.2
R1L	9.4	1.1	R1L	9.6	1.5	R1L	12.7	2.9
R2R	6.9	1.3	R2R	8.9	1.8	R2R	12.2	2.5
R2L	13.9	0.9	RsL	5.4	0.5	R2L	12.3	2.5
R3R	9.5	0.6	R3R	4.8	0.5	R3R	10.7	1.7
R3L	11.2	0.6	R3L	10.1	1.2	R3L	5.5	1.1
R4R	15	1.3	R4R	8.9	0.8	R4R	7.8	1.2
R4L	6.8	0.4	R4L	10.7	1.8	R4L	7.8	2.5
R5R	10.2	0.6	R5R	14.6	0.5	R5R	7.7	1.2
R5L	7.9	0.4				R5L	9.2	1.7
R6R	8.8	0.6						
R6L	9.7	0.9						
MEAN ± S.E.	10.16 ± 0.75	0.78 ± 0.092	MEAN ± S.E.	8.4 ± 1.2	1.14 ± 0.19	MEAN ± S.E.	9.59 ± 0.76	1.85 ± 0.22

Table 6.3

Individual NA and DA values of carotid bodies removed from rats killed by exposure to microwave irradiation (M.W.) for 30 seconds, microwave when rats were anaesthetised and microwave irradiation after cervical dislocation.

Values given as p mol/carotid body.

and 6.2 (see Figure 6.1)1. A similar effect was recorded in the superior cervical ganglion. Thus in five S.C.G. from rats exposed to microwave irradiation after cervical dislocation the mean values of NA was 35.44 +/- 3.41 and for DA was 2.92 +/- 0.32 p mols/ganglion (= mean +/- S.E.) as compared to 185.76 +/- 5.84 and 21.45 +/- 0.74 p mols (mean +/- S.D.) in ganglia removed after cervical dislocation.

COMPARISON OF THE RESULTS

Table 6.4 shows the mean values for NA and DA in the carotid bodies obtained by the different methods.

The NA mean value of carotid bodies removed from anaesthetised rats was not significantly different from that of rats killed by cervical dislocation ($p > 0.5$, Student's 't' test), nor was the DA mean value significantly different ($0.5 > p > 0.1$, Student's 't' test).

However, exposure of the rats to microwave irradiation either alone or preceded by anaesthesia or cervical dislocation, led to approximately a 40% reduction in the mean value of NA level and more than 80% reduction in the DA level per carotid body, as compared to those of carotid bodies removed under anaesthesia or after cervical dislocation.

6.1.2. DISCUSSION

FIGURE 6.1 ILLUSTRATES THE NORADRENALINE AND DOPAMINE
LEVEL OF RAT CAROTID BODIES REMOVED FROM
ANIMALS SUBJECTED TO DIFFERENT CONDITIONS.

THE CAROTID BODIES REMOVED FROM ANIMALS:

- A - UNDER ANAESTHESIA (N = 12)
- B - KILLED BY CERVICAL DISLOCATION (N = 12)
- C - KILLED BY MICROWAVE IRRADIATION
(N = 12)
- D - ANAESTHETISED THEN KILLED BY MICROWAVE
IRRADIATION (N = 9)
- E - KILLED BY CERVICAL DISLOCATION FOLLOWED
BY MICROWAVE IRRADIATION (N = 10)

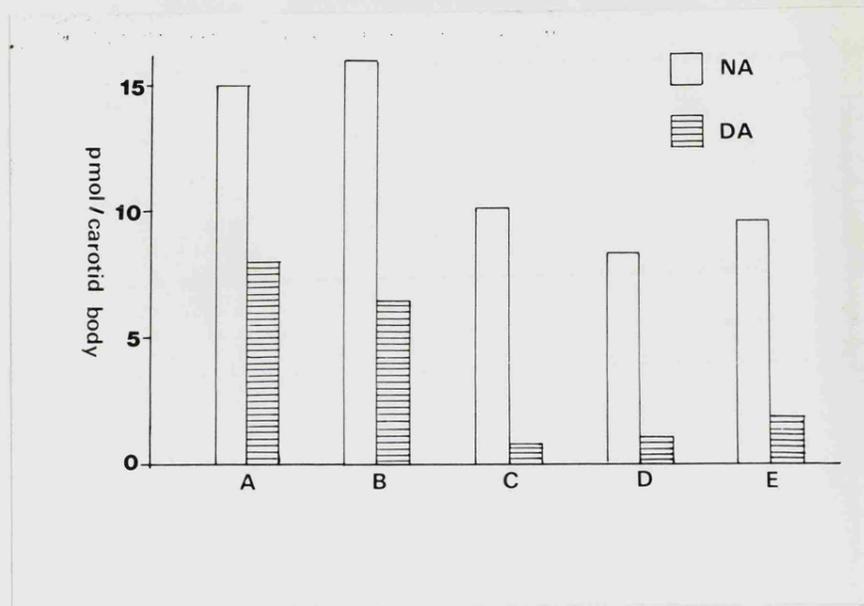


FIGURE 6.1

TREATMENT	NORADRENALINE	DOPAMINE	NO. OF OBSERVATIONS
Anaesthesia	15.06 ± 1.73	7.98 ± 0.85	12
Cervical Dislocation	16.01 ± 1.31	6.48 ± 0.97	12
Microwave Irradiation	10.16 ± 0.75	0.78 ± 0.09	12
Microwave Irradiation + Anaesthesia	8.4 ± 1.2	1.14 ± 0.19	9
Microwave Irradiation After Cervical Dislocation	9.59 ± 0.76	1.85 ± 0.22	10

Table 6.4

NA and DA content in carotid bodies of rats treated by different methods.

Values given as: mean ± S.E. p mol/carotid body.

This study of the endogeneous catecholamine content in the rat carotid body revealed higher NA than DA in marked contrast to the results reported by Hellstrom et al (1975, 1977b, 1978).

Since, in most of Hellstrom's work the rats were killed by cervical dislocation or under anaesthesia, the levels of catecholamines were also determined after these procedures. Those obtained here after killing the rats by cervical dislocation showed that there was no significant differences in the levels of NA and DA as compared to the anaesthetised rats.

One factor which may be important is the time required for removal of the organs. In this respect it took longer to remove the carotid bodies from anaesthetised rats than from animals killed by cervical dislocation; this is largely because of the lack of haemorrhage in the dead animal, and hence less care is needed in dissection.

In this study microwave irradiation significantly reduced the concentration of both NA and DA. In the caudate nucleus of the rat Sharpless and Brown (1978) reported a considerable reduction in DA levels, after microwave irradiation; the NA was not significantly reduced. The exposure time (6 seconds), and microwave output (1.3 kw) used, was less than that used in this work. They also confirmed the suggestion of Lenox et al (1976) and Medina et al (1975) that the heat generated during microwave irradiation could disrupt cell membranes and cause diffusion of substances into the surrounding brain tissue. In this work, a

jelly-like substance found near the carotid artery bifurcation in irradiated rats was analysed and shown to contain large amounts of NA and DA. This finding supports the suggestions by these authors concerning cell membrane disruption caused by microwave irradiation with consequent diffusion of catecholamines into the surrounding areas.

Whilst microwave irradiation caused in excess of an 80% reduction in DA levels, NA levels were reduced by only some 40%. This difference in the effect of microwave irradiation could be due to variations in amine resistance to heat generated by the procedure. However, this is unlikely as effects on NA and DA levels in the S.C.G. (reduction of 80% and 85% respectively) were similar. The more likely explanation in the rat carotid body is that NA and DA are stored in different cells which respond differently to the irradiation (see below effects of sympathectomy).

In this study, the level of carotid body NA was always higher than that of DA. This is a similar finding to those of Mills et al (1978) and Nahorski, Cook, Pallot and Al-Neamy (1980) in the cat carotid body, but in contrast to the results of Hanbauer and Hellstrom (1978), Hellstrom and Koslow (1975) and Hansen and Christie (1981) in the rat carotid body (see Chapter 7).

The variability of the individual values for catecholamine levels in carotid bodies of different rats and of the two sides within the same animal, emphasises the importance and need for

the use of large numbers of carotid bodies to achieve realistic results.

To this author cervical dislocation is the best method for killing rats to be used for catecholamine level determination in the carotid body, it makes dissection much easier, and results in less variability between the two organs of the same animal.

6.2. CATECHOLAMINE CONTENT IN CAROTID BODIES OF RATS OF DIFFERENT STRAINS AND OF SPONTANEOUSLY HYPERTENSIVE RATS

6.2.1. CATECHOLAMINE CONTENT IN CAROTID BODY OF DIFFERENT STRAINS OF RATS

The individual carotid body levels of NA and DA in three strains of rats (Sprague-Dawley, Leicester-Wistar and Normotensive Wistar-Kyoto) were measured in this study.

Table 6.5 shows the mean NA and DA values in the carotid bodies of the three strains of rats. Both Sprague-Dawley and Leicester-Wistar strains have higher NA than DA levels by a factor of some 50%. The DA levels of both strains were similar, whilst the NA level of the Sprague-Dawley strain is higher than that of the Leicester-Wistar strain. However, in the normotensive Wistar-Kyoto strain the DA levels were about 50% higher than the NA levels, the latter being similar to that in Leicester-Wistar rats. The DA level, however, is more than two-fold higher.

RAT'S STRAIN	NA	DA	NO.
SPRAGUE-DAWLEY	16.51 ± 1.32	7.16 ± 0.7	22
LEICESTER-WISTAR	12.25 ± 0.7	7.71 ± 0.65	24
WISTAR-KYOTO	12.59 ± 1.3	17.32 ± 1.02	33

Table 6.5

Mean NA and DA values in carotid bodies of different strains of rats.

Values given as Mean (p mol/carotid body) ± standard error.

No. = Number of carotid bodies used in the estimation.

6.2.2. CATECHOLAMINE CONTENT IN CAROTID BODIES OF SPONTANEOUSLY HYPERTENSIVE (SHR) RATS

Twelve carotid bodies from six spontaneously hypertensive Wistar-Kyoto rats, were used in this study. The individual NA and DA values of these carotid bodies are shown in Table 6.6

There was great variability in the NA and DA contents in different organs; the NA and DA mean values being 30.22 ± 4.03 and 20.87 ± 1.67 p mols/carotid body (mean \pm S.E.) respectively.

Table 6.7 shows the mean NA and DA values of the normotensive Wistar-Kyoto rats (considered as controls), and the hypertensive rats of the same strain. The hypertensive rats had a significantly higher NA level than the normotensive organs ($p < 0.001$, Student's 't' test). Although the mean DA values were also higher in hypertensive rats, they were not significantly so ($0.1 > p > 0.05$, Student's 't' test).

6.2.3. DISCUSSION

The catecholamine concentration in carotid bodies of different strains of rats, killed by cervical dislocation as shown in Table 6.5, demonstrated the presence of a significant variation of catecholamine levels in different strains of rats. In Sprague-Dawley and Leicester-Wistar rats, the NA levels were considerably higher than DA; only in the Wistar-Kyoto (normotensive) strain was the reverse the case.

C.B. NO.	NA	DA	C.B. NO.	NA	DA
R1R	22.2	21.3	R4R	56.8	22.7
R1L	15.7	20.0	R4L	25.2	10.7
R2R	36.9	30.0	R5R	21.1	23.3
R2L	42.4	27.7	R5L	13.9	22.7
R3R	39.8	16.7	R6R	18.6	24.0
R3L	47.7	11.3	R6L	22.3	20.0

Table 6.6

Individual CA values from hypertensive (Wky) rat carotid bodies.

Value: p mol/carotid body.

WISTAR-KYOTO	NA	DA	N
Normotensive	12.59 ± 1.3	17.32 ± 1.02	33
Hypertensive	30.22 ± 4.03	20.87 ± 1.67	12
'p' values	p < 0.001	0.1 > p > 0.05	

Table 6.7

Mean CA values of normotensive and hypertensive (WKY) rats.

Values given as: mean (p mol/carotid body) ± S.E.

'p' values: Student's 't' test.

n = Number of observations

The results of this study with regard to the carotid bodies of the Sprague-Dawley and Leicester-Wistar rats, are at variance with results reported by other workers e.g. Chiochio et al (1981), Hanbauer and Hellstrom (1978), Hellstrom and Koslow (1975) Hellstrom (1977b), all of whom reported that, in the rat carotid body, DA rather than NA is the predominant amine. Although it remains possible that there are discrete differences in the dissection procedures used in this work and those of Hellstrom, a further difference could lie in the analytical methods employed. In previous work we reported (Mir et al, 1982) identical results obtained by two highly sensitive and specific assays, each dependent upon very different principles; (a) the radio enzymatic assay (Saller & Zigmond, 1978) and the HPLC/ED. The technique of Mass Spectrometry used by Hellstrom et al (1975, 1977, 1978) requires a lyophilization step with acid and it remains possible that release of free dopamine from the conjugated form of this amine present in plasma contained within the carotid body could, in part, result in the differences obtained. This possibility certainly requires further examination. Recently, however, other workers (Hansen & Christie, 1981) using HPLC/ED have reported a dopamine content higher than that of noradrenaline in the rat carotid body, though the large variation between animals and the small number of samples used in their study makes the significance of the results difficult to assess. Furthermore Chiochio et al (1981), using a radio enzymatic assay reported an almost identical level of these amines in the rat carotid body.

It would seem that these variations could relate, in part, to difficulties in complete dissection of the carotid body cleanly from its surrounding nervous tissue. In this context, it is well established that post-ganglionic sympathetic efferents containing noradrenaline traverse the ganglio-glomerular nerve and it may well be that the higher levels of noradrenaline observed in the present study reflect carotid body contamination by this nerve during dissection.

Although, using only Sprague-Dawley rats of comparable size in all his studies, Hellstrom reported markedly different levels of NA in two different publications, whilst the DA levels in these two studies were remarkably constant (see Table 1.1).

If, as suggested by Hess (1978), the NA present in the rat carotid body may be located in autonomic nerves, fibres and occasional ganglion cells, the NA level variation in different studies could be attributed to the surgical methods used in the experiment and to what extent the nearby tissues could be separated from the carotid body before its removal. In this respect, it must be reiterated that in this study the superior cervical ganglion was always removed prior to excising the carotid body.

The carotid body of the spontaneously hypertensive rat contains more NA than that of normotensive rats of the same strain. Whether this is due to primary genetic factors, or is secondary to the hypertension, is not apparent from this study. Gernadt et al (1946) reported that the peripheral chemoreceptors

discharge was remarkably reduced when the blood pressure was raised by large doses of adrenaline, and that local injection of adrenaline into the carotid body circulation had no such effect. These results suggest that the reduction of chemoreceptor discharge was due to improved blood flow through the chemoreceptors caused by the rise in cardiac output and systemic pressure.

The mere fact that carotid body catecholamines are changed in hypertensive rats is of some interest in view of the recent results of Trzebski (1983) and the suggestions of Przybylski (1981) and Przybylski et al (1980, 1981) that changes in arterial chemoreceptor activity may be the primary cause of some cases of hypertension.

The results reported here raise more questions than answers. Thus they offer no insight into the primary cause of the increased NA levels; for example are they due to an increased post-ganglionic sympathetic innervation or to more NA containing Type I cells. Furthermore the study of experimentally induced hypertension (e.g. by salt loading) could provide some interesting observations and insight into the general problem.

6.3. EFFECT OF CHEMICAL AND SURGICAL SYMPATHECTOMY ON THE CATECHOLAMINE CONTENT OF THE RAT CAROTID BODY

6.3.1. CHEMICAL AND SURGICAL SYMPATHECTOMY

In this study:

(a) thirteen adult Sprague-Dawley rats which had undergone unilateral superior cervical ganglionectomy two weeks prior to sacrifice;

(b) six animals of the same strain, treated with the neurotoxin 6-hydroxydopamine (6-OHDA).

were used (for 6-OHDA dose regime see Chapter 2). In addition, from rats treated with 6-OHDA, the S.C.G. and portions of left ventricle from ten animals were examined.

Table 6.8 shows the effect of chemical and surgical sympathectomy on the catecholamine content of the rat carotid bodies as compared to the controls. It also shows the effect of 6-OHDA on catecholamine content in the S.C.G. and heart muscle.

In surgically sympathectomised rats, unilateral ganglionectomy resulted in a loss of more than 90% of noradrenaline content, but no change in dopamine content of the carotid body was noted when compared to the intact side.

Chemical sympathectomy resulted in a 70% reduction in noradrenaline content of the carotid body, whereas dopamine levels were unaffected. The dosage effectiveness of 6-OHDA was confirmed by the 93% reduction in cardiac noradrenaline levels. In addition, the superior cervical ganglia appeared to be totally resistant to the neurotoxin as reported previously (Kostrzewa and Jacobowitz, 1974).

	TISSUE	TREATMENT	NA	DA
SURGICAL SYMPATHECTOMY	CAROTID BODY	INTACT CONTROL SIDE (13)	14.6 ± 2.4	8.1 ± 1.6
		GANGLIONECTOMISED SIDE (13)	1.4 ± 0.4(p)	8.2 ± 1.8
CHEMICAL SYMPATHECTOMY	CAROTID BODY	CONTROL (12)	14.1 ± 2.2	8.7 ± 1.4
		6-OHDA TREATED (12)	4.6 ± 0.8(p)	7.7 ± 1.8
	SUPERIOR CERVICAL GANGLION	CONTROL (12)	115.0 ± 13.7	15.7 ± 1.9
		6-OHDA TREATED (12)	113.8 ± 10.7	15.4 ± 1.3
	HEART (VENTRICLE)	CONTROL (10)	8.1 ± 1.6	-
6-OHDA TREATED (10)		0.6 ± 0.2(p)	-	

Table 6.8

Showing the effect of surgical and chemical sympathectomy on the carotid body and various organs catecholamine content of Sprague-Dawley rats.

Values expressed as p mol/carotid body, p mol/superior cervical ganglion or p mol/mg protein of heart tissue. Mean ± S.E.

Number of determinations given in parantheses.

(p) indicates values statistically different from control (p < 0.05, Student's 't' test).

6.3.2. THE TIME FACTOR EFFECT ON CAROTID BODY CATECHOLAMINE CONTENT IN SURGICALLY SYMPATHECTOMISED RATS

In this study two groups of twelve and nine normotensive Wistar-Kyoto rats were used. Unilateral ganglionectomy was performed (a) in Group I two weeks and (b) in Group II six weeks, prior to sacrifice by cervical dislocation and subsequent removal of the carotid bodies (see Chapter 2 for surgical procedure of ganglionectomy). The results of this work, given in Table 6.9, reveal that two weeks after ganglionectomy there is a reduction of 65% in the noradrenaline content of the ganglionectomised carotid body, whilst after six weeks the reduction is only 35%. In Group II, a neuroma near the carotid body was routinely found. These neuromata were removed and analysed for catecholamines; they were found to contain 70.57 ± 13.5 p mol noradrenaline and 11.58 ± 1.34 p mol dopamine/neuroma (mean \pm S.E., N = 4).

6.3.3. DISCUSSION

Results from unilateral ganglionectomy studies show that most, if not all, NA resides in the sympathetic nerve terminals association with the rat carotid body. This confirms a recent report by Chiochio et al (1981) of a 75% NA loss following ganglionectomy. There is substantial evidence of a post-ganglionic sympathetic innervation of the blood vessels to the rat carotid body (McDonald, 1977b). Our results are also consistent with those of Bolme et al (1977) who were unable to demonstrate any significant dopamine-beta-hydroxylase immunoreactivity in rat Type I cells. In addition, Hess (1978)

TREATMENT	NA	DA
Control (12)	11.95 ± 1.29	15.51 ± 1.78
Sympathectomy 2 weeks (8) (Ganglionectomy)	4.38 ± 1.03 (p)	16.19 ± 3.2
Control (9)	12.69 ± 1.23	17.00 ± 2.93
Sympathectomy 6 weeks (9) (Ganglionectomy)	8.37 ± 1.5 (p)	15.66 ± 2.51

Table 6.9

Effect of time factor on catecholamine content levels in the carotid bodies of surgically sympathectomised rats (1 WKY).

Values given as mean ± S.E. p mol/carotid body.

Number of determinations given in parantheses.

(p) indicates values statistically different from the controls (p < 0.05).

concluded from a histochemical study that the NA content of the rat carotid body was restricted to autonomic nerve fibres.

Results of catecholamine investigation into contents of the rat carotid body following treatment with neuro toxin 6-OHDA, shows that the noradrenaline content is reduced by some 40%. The lower reduction in NA levels produced by this compared with sympathectomy is presumably due to a partial resistance of the post-ganglionic nerve endings. The lack of effect of this agent on DA levels suggests that this amine is probably located in the Type I cells, which are resistant to 6-OHDA treatment as reported by Zuazo and Zapata (1978). Hansen and Ord (1978), in their morphological study reported that high doses of 6-OHDA have no effect on Type I cells and they suggest that this may be due to either uptake selectivity of the cells or that Type II cells may provide an effective barrier against such uptake.

In view of these results, it is difficult to explain the observations of Hellstrom et al (1975, 1977) who found neither sympathetic denervation nor chemical sympathectomy to have any effect on catecholamine levels in the rat carotid body. As regards the surgical sympathetic denervation of the carotid body Hellstrom et al (1975, 1977) sectioned the ganglio-glomerular nerves, hence there is a possibility of re-innervation of the carotid body by fibres regenerating from the superior cervical ganglion. In support of this, a later study by Hanbauer and Hellstrom (1978) showed a 50% reduction of NA content in rat carotid body five to seven days after ganglionectomy. Furthermore, Hellstrom (1975, 1977b) and Hellstrom and Koslow

(1975) used long (20 day) post-operative times and this increased the likelihood of re-innervation.

The time factor effect on the carotid body catecholamine content reported in this work makes restoration of the sympathetic innervation to the carotid body a clear possibility.

The lack of effect of 6-OHDA on NA levels in the rat carotid body reported by Hellstrom et al (1975, 1977) may be due to their dosing regimes, (20, 16 and 12 days prior to sacrifice) which seem inadequate to cause irreversible damage. At present there is no other explanation for the differing results obtained by the same laboratory.

CHAPTER SEVEN

CHAPTER 7

THE EFFECTS OF HYPOXIA, HYPEROXIA AND HYPERCAPNIA ON THE CATECHOLAMINE CONTENT OF THE RAT CAROTID BODY

Introduction

In this study, different strains of rats, together with different sources of the same strain, were used; animals were exposed to differing gas mixtures for 30 or 60 minutes by breathing them either spontaneously in a normobaric chamber, or via a positive pressure ventilator. In preliminary experiments for each of the test gases blood gas tensions and pH analyses were performed (see Chapter 2). Table 7.1 shows the results of blood gas tensions and pH analyses for rats ventilated with different gas mixtures. The table illustrates that under conditions of ventilation with 5% O₂ in the normobaric chamber (animal anaesthetised but breathing spontaneously) the animals were not only hypoxic but also hypercapnic. This presumably reflects the hyperventilation induced by this marked stimulus and explains why many animals died during exposure to 5% O₂.

7. 1. THE EFFECTS OF BREATHING HYPOXIC GAS MIXTURES ON THE RAT CAROTID BODY CATECHOLAMINE CONTENT

7. 1. 1. THE EFFECTS OF BREATHING 5 % O₂ IN 95 % N₂ FOR 30 MINUTES

N	METHOD	GAS USED	DURATION	Pa O ₂ (torr)	Pa CO ₂ (torr)	pH
5	N.B.C.	ROOM AIR	0 min	124.4 ± 5.4	34.4 ±0.76	7.4 ±0.01
5	N.B.C.	ROOM AIR	30 min	124.1 ± 5.7	34.8 ±1.2	7.37 ±0.02
3	PUMP	ROOM AIR	30 min	130.0 ± 1.2	30.3 ±0.88	7.45 ±0.02
6	N.B.C.	5% O ₂ in N ₂	30 min	48.3 ± 1.9	22.1 ±2.0	7.12 ±0.03
4	PUMP	5% O ₂ in N ₂	30 min	45.8 ± 1.7	21.0 ±2.7	7.24 ±0.02
5	N.B.C.	10% O ₂ in N ₂	30 min	65.3 ± 2.0	31.1 ±1.7	7.30 ±0.04
12	PUMP	10% O ₂ in N ₂	30 min	59.7 ± 1.5	31.0 ±1.3	7.41 ±0.02
5	PUMP	10% O ₂ in N ₂	60 min	58.8 ± 1.3	30.8 ±1.3	7.41 ±0.03
5	N.B.C.	100% O ₂	30 min	374.0 ±11.3	32.8 ±2.7	7.32 ±0.02
7	PUMP	100% O ₂	30 min	507.7 ±20.8	31.4 ±1.6	7.44 ±0.01
3	N.B.C.	5% CO ₂ in AIR	30 min	121.7 ± 1.2	42.2 ±0.6	7.27 ±0.04
6	PUMP	5% CO ₂ in N ₂	30 min	131.6 ± 5.7	70.0 ±1.3	7.19 ±0.03

Table 7.1

PaO₂, PaCO₂ and pH for rats ventilated in anormobaric chamber (N.B.C.) or by ventilator (Pump), with different gas mixtures or room air for different times.

Values given as mean ± Standard Error.

N = number of animals used.

Two groups of rats were exposed to 5% O₂ in N₂ for 30 minutes; one group was placed in an environmental chamber, the other was artificially ventilated. The results are illustrated in Table 7.2.

The hypoxic stimulus did not have any significant effect on the noradrenaline content of the carotid body in either group. In contrast dopamine content in both groups was significantly reduced ($p < 0.01$, Student's 't' test, in both conditions).

In the chamber group the reduction of dopamine as a percentage of the control was 36%, while in the artificially ventilated group it was 38%; the two mean values are not, however, significantly different.

7. 1. 2. THE EFFECTS OF BREATHING 10% O₂ IN 90% N₂ FOR 30 AND 60 MINUTES

A - Breathing 10% O₂ in 90% N₂ for 30 minutes

In this study, four groups of rats were used; in the first group (Table 7.3.A) were five controls and ten hypoxic (10% O₂ in 90% N₂ for 30 minutes) anaesthetised Sprague-Dawley (S.D./L) rats - the animals breathed the test gas spontaneously in the chamber.

The other three groups were artificially ventilated (Table 7.3.B) with the same hypoxic mixture for 30 minutes.

CONDITION	NA	DA	Pa'O ₂ (torr)	PaCO ₂ (torr)	pHa
A -					
CONTROL (8)	14.1 ± 1.1	7.9 ± 0.85	124 ± 5.4	34 ± 0.76	7.4 ± 0.01
HYPOXIC (20)	12.9 ± 0.82	*5.0 ± 0.28	48 ± 1.9	22 ± 2.0	7.12 ± 0.03
B -					
CONTROL (14)	13.3 ± 0.5	8.1 ± 0.35	130 ± 1.2	30 ± 0.88	7.4 ± 0.02
HYPOXIC (12)	12.9 ± 0.65	**5.0 ± 0.43	45.8 ± 1.7	21.0 ± 2.7	7.24 ± 0.02

Table 7.2

The effect of breathing hypoxic gas mixture (5% O₂ in 95% N₂) for 30 minutes on the rat (Sprague-Dawley (L)) carotid body catecholamine content.

Rats were breathed:

A - spontaneously;

B - artificially

Values given as mean ± S.E. p mol/carotid body.

Number of observations given in parentheses.

* Value statistically different from control (0.01 > p > 0.001, Student's 't' test)

** Value statistically different from control (p < 0.001, Student's 't' test)

CONDITION	RAT STRAIN	NO. OF OBS.	NA	DA
A -				
CONTROL	S.D./L	10	11.21 ±1.1	8.63 ±0.5
HYPOXIC	S.D./L	20	12.8 ±0.81	5.6* ±0.48
B -				
1-CONTROL	S.D./L	20	14.0 ±0.7	7.0 ±0.48
HYPOXIC	S.D./L	26	13.4 ±0.64	5.7** ±0.31
2-CONTROL	S.D./B + K	11	11.7 ±1.53	7.8 ±1.0
HYPOXIC	S.D./B + K	20	11.9 ±0.51	4.94*** ±0.33
3-CONTROL	W/L	20	13.6 ±0.6	6.5 ±0.39
HYPOXIC	W/L	20	13.0 ±0.68	4.7* ±0.27

Table 7.3

The effect of breathing hypoxic gas mixture (10% O₂ in 90% N₂) for 30 minutes on the catecholamine content of the carotid bodies of different strains of rats.

Values given as: mean ± S.E. p mol/carotid body

p value (Student's 't' test):

* p < 0.001

** 0.05 > p > 0.02

*** 0.02 > p > 0.01

Rats were breathed:

A - Spontaneously

B - Artificially

Again, the rats were Sprague-Dawley (L, and B + K colonies) and Wistar (Leicester colony).

In all four groups carotid body noradrenaline levels were not significantly different from control values whereas dopamine levels were greatly reduced as compared with control values ($p < 0.02$, to $p < 0.001$, Student's 't' test). The dopamine reductions range from 18% to 36% from control levels in the four groups studied.

B - Breathing 10% O₂ for 60 minutes

This study used six control and five experimental (Sprague-Dawley, B + K) rats. The control rats were ventilated with room air, while the experimental animals received 10% O₂ in 90% N₂ - both for 60 minutes (see Table 7.4.B).

As in the other hypoxic rats, the carotid body noradrenaline contents were similar in control and experimental situations, while the dopamine contents were significantly reduced ($p < 0.001$, Student's 't' test). The percentage dopamine reduction was 35.

7. 1. 3. COMPARISON OF RESULTS OF HYPOXIC RATS

These results confirm the observations of Hellstrom and Koslow (1975), Hellstrom (1977), Hanbauer and Hellstrom (1978) that hypoxia causes a selective depletion of carotid

CONDITION	NA	DA	% DA REDUCTION
A - CONTROL (20)	11.7 ± 1.53	7.8 ± 1.0	36%
HYPOXIC (20) (30 min.)	11.9 ± 0.51	4.94* ± 0.33	
B - CONTROL (12)	11.9 ± 1.3	8.68 ± 1.3	35%
HYPOXIC (10) (60 min.)	12.5 ± 0.74	5.65** ± 0.44	

Table 7.4

Effect of duration of breathing hypoxic gas mixture (10% O₂ in 90% N₂) on the catecholamine content of the rat carotid body (S.D./B + K)

A - 30 minutes

B - 60 minutes

Values given as mean ± S.E. p mol/carotid body.

p values Student's 't' test:

* 0.02 > p > 0.01

** p < 0.001

body dopamine content. The reduction in percentage terms after 30 and 60 minutes of 10% O₂ in 90% N₂ are remarkably similar (36 and 35% respectively) (see Table 7.4.).

The severity of hypoxic stimuli (5 and 10% O₂) seems not to affect the amount of reduction in dopamine levels in both spontaneously and artificially breathed animals ($p > 0.05$, Student's 't' test) (see Table 7.5.).

7. 2. THE EFFECTS OF HYPEROXIA ON THE CATECHOLAMINE LEVELS OF THE RAT CAROTID BODY

In this investigation two groups of rats (S.D./L) were subjected to 100% O₂ for a period of 30 minutes. The first group of animals breathed the gas spontaneously in the normobaric chamber; the second group were ventilated via the Harvard Type Respirator (Table 7.6. A and B).

The noradrenaline level mean value of the two groups was not affected by the stimulus when compared to their control ($p > 0.5$, Student's 't' test); the dopamine content, however, was significantly increased ($p < 0.001$ in the chamber group and $0.05 > p > 0.02$ in the artificially ventilated rats, Student's 't' test). The dopamine increases in the two groups (Table 7.6 A and B) was 39% and 56% respectively as compared to the control levels.

CONDITION	NO. OF OBS.	DA	'p' VALUE
SPONTANEOUSLY BREATHED			
5% O ₂ in N ₂	(20)	5.0 ± 0.28	p > 0.05
10% O ₂ in N ₂	(20)	5.6 ± 0.48	
ARTIFICIALLY BREATHED			
5% O ₂ in N ₂	12	5.0 ± 0.43	p > 0.05
10% O ₂ in N ₂	26	5.7 ± 0.31	

Table 7.5

Effect of the severity of hypoxic stimulus (5% and 10% O₂ in N₂) on the dopamine content of the rat carotid body.

Values given as: mean ± S.E. p mol/carotid body.

'p' value: Student's 't' test.

CONDITION	RAT STRAIN	NA	DA
A - CONTROL (10)	S.D./L	12.6 ± 1.27	8.18 ± 0.77
HYPEROXIC (20)	S.D./L	12.8 ± 0.87	11.4 ± 0.78*
B - CONTROL (20)	S.D./L	14.0 ± 0.7	7.0 ± 0.48
HYPEROXIC (12)	S.D./L	13.2 ± 1.31	10.94 ± 1.64**

Table 7.6

Effect of breathing hyperoxic gas (100% O₂) for 30 minutes on the rat carotid body catecholamine content.

The rats were breathed:

A - Spontaneously

B - Artificially

Values given as: mean ± S.E. p mol/carotid body.

'p' values, Student's 't' test, compared to control:

* p < 0.001

** 0.05 > p > 0.02

7. 3. THE EFFECTS OF BREATHING HYPERCAPNIC GAS (5% CO₂ IN AIR FOR 30 MINUTES ON THE CATECHOLAMINE CONTENT OF THE RAT CAROTID BODY

In this study four groups of rats were used. The first group was left to breathe 5% CO₂ in air for 30 minutes in the chamber. The other three groups were ventilated with the same gas mixture artificially; the data relative to this experiment is shown in Table 7.7 A and B.

Like hypoxia and hyperoxia, the hypercapnia had no significant effect on the noradrenaline content in any of the experimental situations. Hypercapnia, however, led to a significant increase in the dopamine mean values in all four groups. The level of significance of these increases was $p < 0.01$ or $p < 0.001$, Student's 't' test (see Table 7.7).

In the percentage term the dopamine increases in the four groups used compared to their controls was 21%, 37%, 52% and 49% respectively.

7. 4. EFFECTS OF CUTTING THE CAROTID SINUS NERVE ON THE DOPAMINE CONTENT OF THE RAT CAROTID BODY UNDER HYPERCAPNIC CONDITIONS

In this investigation, six rats (Sprague-Dawley/B + K) were ventilated artificially with 5% CO₂ in air for 30 minutes. Prior to the ventilation the carotid sinus nerve was

CONDITION	STRAIN	NA	DA
A -			
1. CONTROL (20)	S.D./L	11.91±0.85	8.41±0.47 *
HYPERCAPNIC (37)	S.D./L	12.65±0.61	10.13±0.78
B -			
2. CONTROL (20)	W/L	13.6 ±0.6	6.5 ±0.39 *
HYPERCAPNIC (15)	W/L	13.98±0.68	8.92±0.6
3. CONTROL (37)	S.D./L	12.1 ±0.55	7.6 ±0.43
HYPERCAPNIC (25)	S.D./L	11.93±0.7	11.6 ±1.0 **
4. CONTROL (18)	S.D./B+K	11.79±1.11	8.39±0.9
HYPERCAPNIC (19)	S.D./B+K	11.36±0.82	12.48±1.0 **

Table 7.7

Effect of breathing hypercapnic gas mixture (5% CO₂ in air) for 30 minutes on the catecholamine content of rat carotid body.

(A = spontaneously; B = artificially breathed)

Values given as: Mean ± S.E. p mol/carotid body.

p value compared to the control (Student's 't' test)

* 0.01 > p > 0.001 or p < 0.01

** p < 0.001

cut on one side whilst the other nerve was kept as the intact control.

Although the dopamine mean values in both the cut and intact side are higher than the previous control values (cut 10.4 +/- 1.84, n = 6; intact 11.7 +/- 1.72, n = 6, mean +/- S.E. p mol/carotid body), they are not significantly different ($p > 0.5$, Student's 't' test).(see table 7.8)

This preliminary experiment suggests that hypercapnia is acting at the level of the Type I cell rather than via any descending influence.

7. 5. DISCUSSION

Table 7.9 summarises the effects of breathing various gas mixtures on the catecholamine level of the rat carotid body.

In this study, the dopamine content of the rat carotid body decreased during exposure to acute short-term hypoxia, while the noradrenaline levels remain unchanged. These results are in agreement with the previous reports of Hanbauer and Hellstrom (1978) and Hellstrom et al (1976). Under similar conditions Mills and Slotkin (1975) found a decrease in noradrenaline content of the cat carotid body.

Hellstrom and Hanbauer (1976, 1978) additionally

CONDITION	DA
S.N. INTACT (6)	11.7 ± 1.72
S.N. CUT (6)	10.4 ± 1.84

Table 7.8

Effect of cutting the C.S.N. on their carotid body catecholamine level in rats (S.D./B+K) breathing hypercapnic gas (5% CO₂ in air).

DA = p = p > 0.5

'p' Student's 't' test

CONDITION	NA	DA
CONTROL (8)	14.1 ± 1.1	7.9 ± 0.85
HYPOXIC (20) (5% O ₂ in N ₂)	12.9 ± 0.82	5.0 ± 0.28*
CONTROL (10)	12.12 ± 1.1	8.63 ± 0.5
HYPOXIC (20) (10% O ₂ in N ₂)	12.8 ± 0.81	5.6 ± 0.48**
CONTROL (10)	12.6 ± 1.27	8.18 ± 0.77
HYPEROXIC (20) (100% O ₂)	12.8 ± 0.87	11.4 ± 0.78**
CONTROL (20)	11.91 ± 0.85	8.41 ± 0.47
HYPERCAPNIC (37) (5% CO ₂ in air)	12.65 ± 0.61	10.13 ± 0.78**

Table 7.9

Effects of breathing different gas mixtures on the rat (Sprague-Dawley/L) carotid bodies catecholamine content. The rats were breathed spontaneously for 30 minutes.

Values given as: Mean ± S.E.

Values statistically different from control (Student's 't' test)

* 0.01 > p > 0.001

** p < 0.001

reported that the decrease in dopamine content was related to the severity and duration of the hypoxic stimulus and was independent of the innervation of the carotid body from either the carotid sinus nerve or the superior cervical ganglion. Mills and Slotkin (1975) found a similar relationship with regard to noradrenaline release.

As regards the effect of severity of the hypoxic stimulus, this study showed that in both spontaneously breathing and artificially ventilated rats the magnitude of the hypoxic stimulus did not result in significant differences. This was true whether 5% and 10% O₂ were used for the same period of time, or whether the effects of 10% O₂ were compared after 30 and 60 minutes.

The interpretation of these experiments is difficult because the dopamine reduction may not be due exclusively to the direct releasing effect of the hypoxic stimulus, but also to secondary factors induced by it. Hence the reduction in content could also be due to a reduction in the synthetic rate of the compound. Davis and Carlsson (1973) reported that hypoxia decreases both the synthesis and metabolism of monoamines in the brain. According to Hanbauer and Hellstrom (1978) there was no change in dopamine turnover during exposure to hypoxia; their data was obtained in experiments in which synthesis was blocked by α -methylparatyrosine. Regrettably the doses used were probably inadequate and insufficient times were allowed after injection of the drug for a stable condition to be produced.

However the same authors did demonstrate that the carotid body effects were not merely non-specific effects of hypoxia for the same stimulus failed to reduce the catecholamine content of the caudate nucleus, superior cervical ganglion and adrenal medulla. In pilot experiments by the present author using the same compound the initial drop in dopamine levels was so rapid as to render interpretation impossible.

Gonzales et al (1981) and Fidone et al (1982a,b) reported on increased turnover of dopamine in the carotid body after exposure to hypoxia. Hence the question of turnover time requires additional work; suffice to say that it has been shown repeatedly in other catecholaminergic structures that transmitter synthesis and release are well controlled in order that content is roughly maintained (e.g. Sedval and Kopin 1967, Roth et al 1967a,b, Murrin & Roth, 1976).

The results of Mir et al (1983) offer an alternative interpretation of the present data. Using a radio ligand binding assay these authors have been able to confirm the results of Digner et al (1982^b) concerning the presence of specific dopamine receptors within the carotid body of the rabbit. By using specific D1 and D2 blocking compounds the former authors have also demonstrated that it is the D2 receptor which accounts for most of the receptor binding. Sinus nerve denervation resulted in a 40% reduction in receptor binding; the likeliest explanation of this finding is that the remaining 60% of receptors are located on some

other tissue element. The Type I cell is the likeliest element. We may postulate that the dopamine secreted from the Type I cells during hypoxia acts at two sites, the afferent nerve ending and the Type I cell membrane. At the nerve ending the dopamine is inhibitory and leads to a reduction in sensory nerve discharge (e.g. McQueen, 1983, Horn et al, 1983). The action at the Type I cell is not established but it is reasonable to suggest that it causes either an increased synthesis of dopamine or a decreased release of dopamine. This hypothesis could be tested by comparing the dopamine levels after hypoxic stimulation in animals treated with D2 dopaminergic blocking agents (e.g. domperidone) prior to the hypoxia stimulus and in control animals treated with the vehicle. If the suggestion is correct, domperidone should lead to a sustained release of dopamine, the magnitude of which is related to the severity of the stimulus.

The increase in dopamine content found after treatment with 100% O₂ for 30 minutes is in disagreement with the results of Hanbauer and Hellstrom (1978) who reported no change after similar exposure. However, in the cat, Mills and Slotkin (1975) found an increase in noradrenaline (the predominant catecholamine) after exposure to 40% O₂.

5% CO₂ for 30 minutes also leads to an increase in dopamine content, the magnitude of this increase being similar to that produced by 100% O₂. No other reports on the effects of CO₂ on dopamine content have appeared.

It is interesting to speculate on the possible mechanisms of these increases in dopamine content. The general rule concerning the catecholaminergic systems in other parts of the body is that stimulation of release leads to stimulation of synthesis (e.g. Roth et al, 1967a,b); as content is increased by 5% CO₂ and 100% O₂ stimulation of synthesis seems an unlikely explanation. A second possibility is that there is an increased uptake of released dopamine under these conditions. The fact that only 30 minutes of ventilation with 100% O₂ or 5% CO₂ leads to a 40% increase in dopamine content makes this unlikely for the synthetic rate would need to be extraordinarily high to cause such an increase. The remaining possibility is that the stimuli inhibit, or totally abolish, release. This mechanism again suggests a rapid synthesis of dopamine. In pilot experiments, referred to above, it was found that dopamine content fell precipitously after injection of alphanethylparatyrosine; such a finding is in keeping with a high turnover rate of dopamine in the rat carotid body.

In summary, hypoxia leads to a reduction in dopamine content of the rat carotid body whilst hyperoxia and hypercapnia increase dopamine content. To establish the mechanism of these actions requires the determination of dopamine turnover times in the carotid body under different conditions. The possible role of dopamine content in the functioning of the carotid body is discussed in Chapter 8.

CHAPTER EIGHT

CHAPTER EIGHT

FINAL DISCUSSION

The quantitative structural studies of cat and rat carotid body tissue have highlighted two potentially important dissimilarities between the organ in the different species - firstly, the greater quantity of specific tissue in the rat carotid body and, secondly, the differences in vesicle size in Type I cells of the two organs. The physiological importance of the former observation is obscure; the second finding probably relates to the differing catecholamines stored in the two species. Concurrent with this study other work from our laboratory has shown dramatic differences in the innervation of Type I cells in cat and rat carotid body (Blakeman, Pallot & Al Neamy, 1983).

The vexing question of sub-types of Type I cells remains only partially answered. The ultrastructural data concerning the rat is unequivocal; no subdivision on the basis of ultrastructural criteria is possible. This conclusion accords with biochemical data that most, if not all, Type I cells store dopamine in the rat (Mir et al 1982). In this regard it must be mentioned that the residual amounts of noradrenaline in the sympathectomy studies (Chapter 6) do not necessarily imply that the compound is stored in Type I cells for there is always the possibility of sympathetic terminals remaining in the carotid body from those fibres known to travel in the sinus nerve (Eyzaguirre & Uchizona 1961); furthermore it is known that a

small number of ganglion cells are found in the rat carotid body (McDonald & Mitchell, 1975).

The position in the cat is more difficult. There are undoubtedly some cells in this species which show a bimodal distribution of vesicle diameters; equally there is no doubt from our biochemical studies that both dopamine and noradrenaline are stored in cat carotid body Type I cells in a ratio of some 1:5 (Mir et al, 1982).

Two interpretations of our data are thus possible. Firstly that those cells with a bimodal distribution histogram store both amines, whilst most cells store only noradrenaline and, secondly, that all cells store both amines, but only in a small number of cells are there sufficient dopamine vesicles to cause a recognisable peak. The data does not permit of a distinction between these possibilities. It is thus imperative that a careful study of dopamine beta hydroxylase (D.B.H.) distribution in the cat carotid body be performed to provide definitive evidence on this point, for D.B.H. catalyses the conversion of dopamine to noradrenaline and hence only those cells synthesising dopamine will contain the enzyme.

The most interesting finding reported here concerns the effects of hypercapnia on the dopamine levels in the rat carotid body, for they may be of importance in the physiological responses of the carotid body. It is known that hypercapnia and hypoxia have a multiplicative effect on carotid body discharge, which is difficult to explain. Now, there is ample evidence that

carbon dioxide and hypoxia act in the carotid body via different mechanisms; it has been shown that uncoupling agents abolish the effects of hypoxia on chemosensory discharge whilst the consequences of carbon dioxide are largely unchanged (e.g. see Lahiri 1983). Thereby an additive effect of hypercapnia + hypoxia is simply explained. The multiplicative effect could be provided by a removal of any tonic inhibitory activity within the organ, in other words if dopamine release is inhibited during hypercapnia then the combined effects of CO₂ and O₂ lack on afferent would be greater than the sum of each stimulus acting alone. Furthermore, part of the stimulatory effect of hypercapnia may be due to reduced dopamine release and hence removal of an inhibitory influence. The hypothesis suggested here is easily demonstrated by examining the effects of combined hypoxia/hypercapnia before and after D2 blocking agents; it predicts that the multiplicative effects of the combined stimuli would be attenuated by D2 blockade. To date this experiment has not been attempted.

In summary this work describes quantitative ultrastructural features of the cat and rat carotid bodies. The major findings are:

- 1) That rat Type I cells are ultrastructurally a homogenous population whilst, in the cat carotid body, there is a possibility of two types of Type I cell.
- 2) In both species there are changes in mitochondrial and EDCV Vv% during exposure to hypoxic stimuli within the physiologic range.

The biochemical studies of catecholamines show:

- 1) That, in the adult Sprague Dawley rat, dopamine is the predominant amine stored in the carotid body.

- 2) That in common with previous reports, hypoxia leads to a specific dopamine depletion.

- 3) That hyperoxia and hypercapnia lead to an increase in dopamine levels in the rat carotid body.

APPENDICES

APPENDIX 1

ROUTINE FIXATION PROCEDURES

PHOSPHATE BUFFER STOCK SOLUTIONS (A + B)

SOLUTION A - 0.1m Sodium Dihydrogen Orthophosphate (m.wt. - 156.01)

SOLUTION B - 0.1m Disodium Hydrogen Orthophosphate Anhydrous (m.wt. 141.96)

Approximately 20 ml solution A + 80 ml solution B give a working solution of pH 7.3

SUCROSE BUFFER WASH

6.84 g Sucrose made up to 100 ml with phosphate buffer - pH 7.3

Wash 3 x 30 mins and leave tissue overnight in fresh buffer at 4°C.

SECONDARY FIXATION

Post-fix with 2% Osmium Tetroxide for 1 hour at 4°C. Wash several times with distilled water.

FIXATION/BEDDING TECHNIQUE

1. Perfusion fix with 0.05% H₂O₂ in 3% Glutaraldehyde in phosphate buffer at pH _{7.2} (100 ml).
2. Perfuse with 3% Glutaraldehyde in phosphate buffer at pH 7.2 (500 ml).
3. Wash 4 x 15 minutes in phosphate buffer.
4. Soak in 2% Osmium Tetroxide (0.1 M phosphate buffer) for 1 hour.
5. Dehydrate in 70% Ethanol for 10 minutes.
6. Dehydrate in 80% Ethanol for 10 minutes.
7. Dehydrate in 90% Ethanol for 10 minutes.

8. Dehydrate in 100% Ethanol for 10 minutes.
9. Dehydrate in Absolute Ethanol for 10 minutes.
10. Incubate with Propylene Oxide (4 x 15 minutes).
11. Incubate in Propylene Oxide and Araldite (1:1) overnight.
12. Incubate in Araldite resin + accelerator (1 drop accelerator to 1 ml resin) in a closed container for 8-12 hours.
13. Embed in Araldite resin + accelerator (1 drop accelerator to 1 ml resin) in a block mould at 60°C for a minimum of 48 hours.

URANYL ACETATE STAIN

10% Uranyl Acetate in Abs. Methanol.

REYNOLDS LEAD CITRATE STAIN

STOCK SOLUTION A

Lead Nitrate Soln - 8.86 g/100 ml distilled water

STOCK SOLUTION B

Tri-Sodium Citrate Soln - 11.53g/100 ml distilled water

For Lead Citrate Stain take:-

15 ml of stock soln A

15 ml of stock soln B

and mix vigorously for 1 minute, then intermittently for 30 mins.

Then add:-

8 ml of 1N* Sodium hydroxide freshly made and filtered - this will clear the solution

Finally add:-

12ml of distilled water - mix by inversion and centrifuge (2000 rpm for 10 mins).

Do not shake the soln after centrifugation, if it is knocked over, re-centrifuge.

All glassware must be scrupulously clean, soaking in dilute nitric acid followed by rinsing in distilled water. CO₂-free water should be used throughout.

STAINING WITH LEAD CITRATE

Stain in a wax-filled petri dish with pellets of NaOH in to remove CO₂ from atmosphere.

Place drop of Stain on wax. Float grid (sections down) on the drop. Replace petri dish lid immediately.

Stain for 4-5 minutes.

Remove grid and wash in CO₂ free, distilled water. Dry on Velin Tissue.

Lead Stain should be taken from the middle of the volume of the bottle. The first drops must be discarded.

The solution must NEVER be put back in the bottle.

APPENDIX 2

REAGENTS

0.1 M Perchloric Acid (P.C.A.)

To 100 ml of distilled water add 0.8 ml of concentrated perchloric acid (caution corrosive/oxidizing). Weigh out 0.8 g of Sodium Metabisulphate and dissolve in 10 ml of distilled water. Add 0.5 ml of this solution to the 100 ml of Perchloric Acid.

Tris Buffer pH 8.6

Weigh out 12 g of Tris and 2 g of EDTA and dissolve both in 100 mls of distilled water. Add conc. Hydrochloric acid dropwise (caution corrosive) until the pH reaches 8.6

Buffers:-

pH 5.2 Acetate/Citrate

5 litres contains

28.7 g Citric Acid H_2O
34.0 g Sodium Acetate $3H_2O$
12.0 g Sodium Hydroxide
1.85g EDTA
0.50g Sodium Octyl Sulphonate (SOS)
500 ml Methanol

NB: Buffer for cation exchange columns is the same except NO methanol or SOS.

APPENDIX 3

During the period of postgraduate study the following papers have been published.

- Al-Neamy, K., Mir, A.K., Pallot, D.J. & Nahorski, S.R. (1981) Isoprenaline-stimulated cyclic A.M.P. formation in the rat carotid body in vivo. *Acta Anat.* 111, 5.
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In addition two papers on the stereology of cat and rat Type I cells are in preparation.

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ACKNOWLEDGEMENTS

I must, first of all, thank the Iraqi Government for its financial support without which this work could not have been carried out.

Dr D.J. Pallot in the Department of Anatomy at Leicester University was my supervisor and it is a pleasure to thank him for his unstinting efforts and valuable assistance whenever required.

I would further like to express my gratitude to Professor F. Beck (Head of Department) for his many acts of kindness.

My thanks must also go to Miss S. Bulman and Mr. T. Owen for their technical assistance in the electron microscopy studies, the considerable and indispensable technical help of Mr. J. Strupish in maintaining the HPLC/ED is very much appreciated and I am grateful to Miss M. Reeve and Miss D. Dolman for their secretarial help.

Finally, my very grateful thanks must go to my wife, Mrs. R. Al-Samman, for her patience, help and encouragement during the course of my studies.

UNIVERSITY OF LEICESTER

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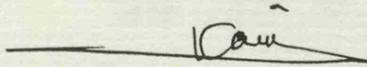
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THE STRUCTURE AND CATECHOLAMINE CONTENT OF THE CAROTID BODY.

A B S T R A C T

The thesis reviews previous work on the ultrastructure and catecholamine content of the carotid body and describes areas of sparse available data.

- 1) The question of sub-populations of Type I cells on the basis of quantitative ultrastructure;
- 2) The reaction of the mitochondria and electron dense-cored vesicles of the Type I cells to physiological hypoxic stimuli;
- 3) The levels and type of catecholamines, stored within the carotid body;
- 4) The influence of sympathectomy on these levels;
- 5) The influence of hypoxia, hyperoxia and hypercapnia on catecholamine levels.

The experimental data obtained has revealed that the rat carotid body contains only one cell population, whilst that of the cat contains two. Both organs show ultrastructural changes in the Type I cells in response to moderate hypoxia.

The catecholamine content studies have shown that the rat stores predominantly dopamine in its Type I cells. In confirmation of previous studies hypoxia is shown to deplete dopamine levels; a new and potentially important finding is that hypercapnia and hyperoxia increase dopamine levels.

The possible significance of these findings is discussed and a testable hypothesis suggested.