Granule-containing Cells of Rat Carotid Body and their Biogenic Amines

An electron microscopic and biochemical study

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av
Rektorsämbetet vid Umeå universitet
för avläggande av medicine doktorsexamen
kommer att offentligen försvaras
i stora föreläsningssalen,
institutionen för anatomi-histologi,
lördagen den 19 april 1975 kl 9.00

av

STEN HELLSTRÖM
Med kand

Norrlands-tryck, Umeå 1975
UMEÅ UNIVERSITY MEDICAL DISSERTATIONS

New Series

No 4

From the Department of Anatomy
University of Umeå, Umeå, Sweden

Granule-containing Cells of Rat Carotid Body and their Biogenic Amines

An electron microscopic and biochemical study

by
Sten Hellström
This thesis is based on the following publications, which will be referred to by their Roman numerals:


IV. Effects of glucocorticoid treatment on catecholamine content and ultrastructure of rat carotid body. Brain Research (submitted for publication). (Together with Stephen H. Koslow.)
INTRODUCTION

The carotid body of mammals, located to the carotid bifurcation, was first discovered in 1743 by Taube. Yet, after more than 200 years of extensive research on the organ (recently reviewed by Biscoe, 1971; Böck, 1973; Kjaergaard, 1973) the morphological and physiological interrelationship is poorly understood. In attempts to establish the nature of the carotid body, different possibilities have been considered. Thus, it has been suggested that it constitutes a sympathetic ganglion (Taube, 1743), an endocrine gland (Luschka, 1862), a sympathetic chromaffin paraganglion (Stilling, 1892; Kohn, 1900) and a parasympathetic non-chromaffin paraganglion (Kose, 1907; Watzka, 1930-31).

The only function confirmed for the carotid body is its capacity as a specific chemoreceptor (de Castro, 1926, 1928; see Heymans and Bouckaert, 1939), the adequate stimuli of which consisted in a reduction of arterial $P_{O_2}$ or pH or an increase in arterial $P_{CO_2}$. Studies by light microscope, chiefly by de Castro (1926, 1928), Hollinshead (1940, 1943) and de Kock (1954, 1960), showed that the carotid body was composed of lobules of cells embedded in richly vascular connective tissue. The organ receives its nerve supply from three sources, namely, the glossopharyngeal and vagal nerves and the superior cervical ganglion. The most numerous cells of the lobules are called Type I (chief, chemoreceptor, glomus) cells. These cells are surrounded by solitary Type II (sustentacular, supporting) cells or their slender-like cytoplasmic extensions.

At the turn of the last century, Stilling (1892) and Kohn (1900) reported that the cells of the carotid body, homologous to adrenal medullary cells, turned brown when treated with chromium salts. Hence the carotid body cells were designated chromaffin cells. Despite doubts of a
true chromaffinity (Adams, 1958; Kose, 1907; Watzka, 1930-31) recent studies by Kobayashi (1971) support the idea that the carotid body cells are chromaffin in nature. Since the work of Gérard, Cordier and Lison (1930) it has been accepted that the chromaffin reaction was caused by oxidation of catecholamines (Pearse, 1972). The observed chromaffinity of the carotid body cells led to the suggestion that the carotid body contained catecholamines.

The introduction of electron microscopy into the field of carotid body research (Engström et al., 1957; Lever, Boyd, 1957; Ross, 1959) did not provide any clue to where the biogenic amines were localized in the carotid body. This technique, however, made it possible to study the ultrastructure of the Type I cells. They were characterized mainly by their electron-dense membrane-bounded granules, generally called granulated or dense-cored vesicles. Similar vesicles were discovered in e.g. sympathetic nerve terminals and adrenal medullary cells and were supposed to store biogenic amines. The Type I cells seemed to be fairly uniform in morphology, although attempts have been made to classify the cells with respect to the dimensions of the dense-cored vesicles (Morita, Chiocchio and Tramezzani, 1969) and with respect to cytoplasmic background electron density (Höglund, 1967; Morita, Chiocchio and Tramezzani, 1969).

The localization of biogenic amines to the Type I cells was definitely confirmed by fluorescence histochemistry (see Böck, 1973; Kobayashi, 1971; Möllmann et al., 1972). In spite of fluoremetrical measurements, much controversy exists with regard to what kinds and amount of monoamines the carotid body stores. The conflicting results have been attributed to differences between species and/or between individuals as well as methodological problems related to the small size of the organ (Kobayashi, 1971; Zapata et al., 1969).
Despite the knowledge that carotid body cells contain biogenic amines, the role of these amines remains unclear. There is, however, evidence against the catecholamines acting as the chemosensory transmitter (see Biscoe, 1971), but notwithstanding they could take part of the chemoreceptor capacity (Zapata, 1975).

Against this background, the aims of the present work on rat carotid body can be summarized as follows:

1. To establish whether or not the carotid body Type I cells are a homogenous group of cells with respect to their ultrastructure, mainly in regard to the granulated vesicles.

2. To study the effects of catecholamine precursors on the Type I cells at the ultrastructural level, particularly on their granulated vesicles.

3. To determine and quantitate the major types of amines stored in the normal adult and infant carotid body.

4. To investigate how the catecholamine levels of the carotid body are influenced by pharmacological agents and denervation experiments, which are known to interfere with the monoamine metabolism.

5. To study effects of glucocorticoids on the carotid body in an attempt to correlate biochemical and morphological data on the granulated vesicles of the Type I cells. Glucocorticoids are known to change the ratio between the levels of different catecholamines in other organs.
MATERIALS AND METHODS

Animals. Male adult (2-months old) Sprague-Dawley rats, weighing 175-250 g, were used. In addition, the determination of catecholamines was performed on 10-day old Sprague-Dawley rats weighing about 25 g.

Electron microscopy

Processing of the tissue. The rats were anesthetized (sodium pentobarbital) and artificially ventilated (gas mixture, 95 % O₂ and 5 % CO₂) before being perfused through the left heart ventricle by ice-cold 1.5 % glutaraldehyde in veronal acetate buffer (pH 7.40, 485 mosm, flowrate 35 ml/min, 15-20 min). The carotid bodies were thoroughly freed from connective tissue and rapidly excised, rinsed in veronal acetate buffer (pH 7.40, 330 mosm, 30 min), postfixed in 1 % ice-cold OsO₄ in the same buffer (1.5 h), rinsed, dehydrated in rising concentrations of acetone and embedded in Vestopal W. Sections were cut at a feeding rate of about 50 nm, placed on grids and stained with 1 % uranyl acetate (37° C, 30 min) followed by lead citrate (20° C, 60 sec). The sections were examined in a Siemens Elmiskop IA at 80 kV. Calibration of the magnification was performed using a carbon grating with 21,575 lines per cm.

Morphometry. Several sections, cut at 4 different levels (about 25 μm apart), were studied from the right carotid body of each rat. From each level 6-10 randomly selected Type I cells (in all 25-35 cells from each carotid body) were examined. To avoid identification problems only such cells were studied which exhibited the nucleus in the sections.

The analysis of diameters of the dense-cored vesicle profiles was performed on paper prints with a magnification of x 48,000 (initial plate magnification x 8,000)
by use of a Zeiss Particle Analyzer TGZ-3. All vesicle profiles of each cell were measured. The vesicles were identified by their dark center surrounded by a loosely fitting triple-layered membrane. Most profiles were circular, but since a few were elongated, an average diameter was determined. The median and mean vesicle profile diameter was calculated for each individual cell and represented in a frequency distribution histogram (one for each rat). To test if the median profile diameters could be separated into different groups, they were exposed to cluster analysis (Edwards and Cavalli-Sforza, 1965; Engelman and Hartigan, 1969). With this test a set of observations may be divided into two (or more) clusters by the calculation of the maximum ratio of the variation between the two groups and that within the groups, thus an estimate was obtained of the optimal distance between two clusters. The maximized ratio was transformed to the level of probability of the partition. The calculations for the cluster analysis were performed by a computer. The mean values of the vesicle diameters obtained represent mean values of the vesicle profiles, but "true" vesicle diameters were calculated according to the empirical method of Froesch (1973).

Determination of volume densities of dense-cored vesicles, mitochondria and nuclei was performed on paper prints with a magnification of x 24,000 (initial plate magnification x 8,000) by the point-counting method (Weibel, 1969). The number of points for the determination of dense-cored vesicles was about 24 per µm² and of the mitochondria and nuclei about 6 per µm². The volume densities were expressed as percentages of the cytoplasmic volume (excluding the nucleus) for vesicles and mitochondria and as percentages of the total cell volume (including the nucleus) for nuclei. Systematic errors due to the Holmes effect (Weibel, 1969) and systematical overestimation of the nuclear volume density (Konwinski and Kozlowski, 1972) were corrected for.
The error of the morphometric procedure was calculated according to Eränkö (1955) by comparing mean values after having repeated the measurement of twenty cells on the electron micrographs. The method error for the volume densities was 2\% for the nuclei, 8\% for the mitochondria and 13\% for the vesicles. For the estimation of cell profile area the error was 2\%. These method errors agree well with the expected relative errors calculated according to Weibel (1963).

An estimate of the volume of the whole cell was calculated from the values of the mean cell profile areas. These estimates are approximations, as the calculations are made under assumptions that the mean cell profile areas represent segments close to the diameter and that the cells are spheres.

**Gas chromatographic - mass spectrometric assay (GC-MS)**

The catecholamines and serotonin were determined by the GC-MS-method described by Koslow, Cattabeni and Costa (1972) and Cattabeni, Koslow and Costa (1972 a), respectively. The rats were killed by a blow on the head and the carotid bodies were rapidly excised. The tissue was homogenized in a solution of ascorbic acid (50 mM) in 0.1 N formic acid (catecholamine determination) or formic acid/acetone (5:95) (serotonin assay). After centrifugation the supernatant was transferred to glass vials. Internal standards, α-methyl-norepinephrine (α-MNE), α-methyl-dopamine (α-MDA) and α-methyl-serotonin (α-M5HT) were added to the supernatant. After drying under a stream of nitrogen the catecholamines were acetylated by the addition of pentafluoropropionic acid (PFPA), dissolved in ethylacetate and heated (60° C, 30 min). Before the injection into the GC-MS (LKB 9000) the excess of PFPA was evaporated and the residue redissolved in ethylacetate.
The following GC-retention times (sec) were registered (column: 3% SE-54 packed on chromosorb G): α-MNE 100, NE 130, E 150, α-MDA 195, DA 265. The serotonin GC-retention times (sec) were (column: 3% OV-17 packed on gas-chrom Q): α-M5HT 150, 5-HT 200.

The fragments (mass-to-charge, m/e) recorded for quantitation of the acetylated compounds were: α-MNE 190, NE 176, E 190, α-MDA 190, DA 176, α-M5HT 438 and 5HT 438. To confirm the specificity of the measurements, the authentic and extracted compounds were analyzed by the technique of multiple ion detection. By measuring two fragments (m/e) the same fragment ratio was obtained for the authentic amines and all endogenous extracted compounds, at their appropriate retention times. The carotid body extracts processed without internal standards did not show any "biological background" at the masses (m/e) and retention times of the internal standards. From the peak heights, representing the ion densities, the concentrations of amines were calculated and presented as pmol/pair of carotid body and/or pmol/mg protein (protein determined according to Lowry et al., 1951). For details about the GC and MS instrumentation, see paper III.

Drugs

L-3,4-dihydroxyphenylalanine (L-Dopa) and reserpine from Sigma Chem. Co., St. Louis, Miss., USA. 5-OH-Dopa and 6-OH-Dopamine as gifts from Hoffmann-La Roche & Co., AG, Basel, Switzerland. Pargyline from Abbot Laboratories, North Chicago, Ill., USA. Diethyldithiocarbamate (DDC)

Footnote: The following abbreviations were used:
DA - dopamine, NE - norepinephrine, E - epinephrine, and 5HT - serotonin.
from Upjohn Co., Kalamazoo, Mich., USA. Dexamethasone (Decadron\textsuperscript{R}) from Merck Sharp & Dohme Int., Rahway, N.J., USA. For the different times of treatment and the injection procedure see original papers II-IV.

Statistics

The significance of the differences between the calculated means was tested by Student's t-test (two-tailed). The difference was considered significant when \( p < 0.05 \).

RESULTS AND DISCUSSION

Morphometric studies of carotid body Type I cells (I)

The cluster analysis revealed two subclasses of Type I cells with respect to the dimensions of their dense-cored vesicles. One subclass of cells (designated \textit{small vesicle cells/SVC}) contained vesicle profiles with a mean diameter of \( 47.4 \pm 0.82 \text{ nm} \) (mean \( \pm \text{SE} \)); in the other subclass of cells (designated \textit{large vesicle cells/LVC}) the vesicle profiles measured \( 63.0 \pm 0.92 \text{ (mean \( \pm \text{SE} \)}) \). The "true" vesicle diameters were about 52 nm and 71 nm respectively, when calculated according to Froesch (1973). There was a significant difference in the volume density of dense-cored vesicles between the SVC and LVC, \( 1.08 \pm 0.102 \% \) vs. \( 1.91 \pm 0.113 \% \) (about 0.4 \% vs. 0.9 \% when corrected for overestimation due to the Holmes effect). When comparing the total cell volumes, an SVC seemed to be about \( 3/4 \) of the size of an LVC. The volumes occupied by nuclei and mitochondria differed between SVC and LVC, for nuclei 52 \% vs. 42 \% and for mitochondria 14.4 \% vs. 16.9 \%. The general morphology of the Type I cells has been extensively described and reviewed earlier (Biscoe, 1971; Böck, 1973). With regard to the two subclasses of Type I cells
reported in this study, it can be added, however, that the finger-like extensions, typical of Type I cells, seemed to be more common for the LVC than for the SVC. Furthermore, the majority of nerve-endings terminated on the LVC. A nerve-ending was defined as a specialized region, separated from the plasma-membranes of the Type I cells by a cleft about 20 nm wide, and exhibiting small agranular vesicles (less than 50 nm).

These findings show that there exist two subclasses of Type I cells with respect to the dimensions of their granulated vesicles. These cells also differ in size, cytoplasmic extensions, and the relative volumes occupied by vesicles, mitochondria and nuclei. If one accepts the concept that the vesicles are stores of biogenic amines, then the different populations of vesicles, each localized to a particular subclass of Type I cells, could suggest that different kinds of monoamines are stored in different kinds of cells. These amines may be involved in various functions of the chemoreceptor capacity (Biscoe, 1971) or perhaps other functions of the organ (Pearse, 1969). Differences in maturity could also explain the presence of two groups of cells.

The ultrastructure of carotid body Type I cells after exogenous administration of monoamine precursors (Ii)

L-Dopa, the physiological catecholamine precursor, and 5-OH-Dopa, a synthetic precursor of biogenic amines (Thoenen et al, 1967), were administered to rats for periods ranging from 15 h. to 60 h. before sacrifice.

5-OH-Dopa markedly increased the electron-density of the granules of the vesicles. In addition the granules almost completely occupied the interior of the vesicles. Many vesicles seemed to accumulate close to the plasma membrane, without any preference for e.g. nerve-endings.
The ultrastructure of Type I cells after L-Dopa treatment resembled that after 5-OH-Dopa administration, except that the change in electron-density was less pronounced. Cluster analysis applied to the diameters of the granulated vesicle profiles showed two populations of vesicles, each localized to separate Type I cells. Compared to those of the control tissue, the vesicle profiles were enlarged (for SVC by about 15% to 55 nm and for LVC by about 22% to 76-78 nm). The volume density of vesicles in both SVC and LVC was increased, chiefly for the LVC. The ratio between the mean cell volume of an SVC to an LVC was about the same as for the controls (roughly 3:4). After L-Dopa treatment for 60 h., however, the mean volume of the LVC was significantly increased, a tendency also observed for the SVC, although not statistically significant.

The observations indicate that the Type I cells possess mechanisms for uptake of amine precursors and synthesis of monoamines. The increase in size and amount of dense-cored vesicles, most pronounced for the LVC, might be interpreted as increased capacities of the vesicles to accumulate monoamines. Obviously these changes did not influence the frequency of the two subclasses of Type I cells. The peripheral localization of vesicles, close to the plasma membrane may point towards an "endocrine-like" function of the vesicles, related to the chemoreceptor capacity.

The biogenic amines of the carotid body (III)

The gas chromatographic - mass spectrometric (GC-MS) assay for quantitation of biogenic amines showed that dopamine (DA) (1953 ± 530 pmol/mg protein; mean ± SE) and norepinephrine (NE) (1142 ± 69.6 pmol/mg protein) are the major monoamines stored in rat carotid body. Serotonin was present at a much lower concentration (506 ± 94.6 pmol/mg protein). Epinephrine could not be detected at all despite a sensitivity of the method of
at least 0.1 pmol. The DA and NE concentration of carotid bodies from 10-day old rats (1067 ± 168 pmol/mg protein and 410 ± 66.3 pmol/mg protein, respectively) was lower than that of the adults.

The concentrations of catecholamines (CA) after surgical as well as chemical (6-OH-DA) sympathetic denervation were unchanged 20 days after the operation. L-Dopa treatment (identical to that described in paper II) and injections of monoamine oxidase inhibitor (pargyline) many-folded the NE and DA concentrations. Relatively, the amount of DA increased more. Reserpine depleted dose-dependently the catecholamines and emptied, almost completely, the amine stores at large doses. Treatment with a dopamine-β-hydroxylase inhibitor (DDC - a metabolite of disulfiram) decreased the NE content, without an expected simultaneous increase in DA.

Though very small amounts of tissue were available (carotid body dry weight: adult 8.3 µg, infant 5.6 µg) it was possible to measure the monoamines of single carotid bodies by the recently developed GC-MS method. In agreement with previous fluorometrical analyses on rabbit and cat (Chiocchio et al., 1971; Dearnaley, Fillenz and Woods, 1968; Zapata et al., 1969) the rat carotid body contained large amount of primarily DA and secondarily NE. Contrary to the findings of Zapata et al (cat) E could not be detected at all. Sympathetic denervation known to decrease the CA content in adrenergic innervated tissues (von Euler and Purkhold, 1951), did not influence the CA content of carotid body. This would lead to the suggestion that the sympathetic nerves terminate upon cells of the vascular bed and do not directly innervate the Type I cells - the assumed stores of CA. Reserpine emptied the stores of CA. In a morphological study on carotid body Type I cells from reserpine-treated rats (Chen, Yates and Duncan, 1969) an electron dense (though decreased) interior of the vesicles was observed. Thus one might suppose that the dark granules,
at least partly, represent a matrix connected with the amine. It is of interest to compare the increased amounts of DA and NE after L-Dopa treatment with the increase in size and volume density of the dense-cored vesicles, as evidenced in paper II. It could be possible that the different populations of vesicles (II) reflect different kinds of stored amines. One might suggest that the amine most increased, DA, is localized to the granulated vesicles with the most increased storage capacities, the larger type of vesicles. Thus, DA and NE would be stored in the different subclasses of Type I cells, LVC and SVC, respectively. This hypothesis is supported by the results obtained from the experiment with DDC. If DA only acts as a precursor of NE, the DA content would have increased when inhibiting the enzyme dopamine-ß-hydroxylase (Cattabeni, Koslow and Costa, 1972 b). In contrast, the DA level was unaffected, which might indicate that the DA present does not, as a whole, serve as a precursor of NE. Most probably NE and DA are stored in different cells. The concentration of serotonin was lower compared to that of CA. Serotonin may be localized to the Type I cells as postulated for the human carotid body by Hamberger, Ritzén and Wersäll (1966). Yet, in fluorescence microscopical studies on the carotid body of mouse and rat by Böck (1973), the only cells exhibiting a typical serotonin fluorescence were mast-cells located in the tissue between the lobules of Type I cells.

**Carotid body of glucocorticoid-treated rats (IV)**

Injections of dexamethasone, a potent synthetic glucocorticoid, in rats resulted in a doubling of the NE (2.6 times) and DA (2.0 times) content of carotid body. E could not be detected at all.

Again, two subclasses of Type I cells were observed, classified according to the dimensions of their granulated vesicles. The subclasses most probably correspond to the observed SVC and LVC of the controls. The diameters of the
vesicle profiles were enlarged by about 20% for SVC and by 10% for LVC. The volume densities of the vesicles increased by factors of 1.8 and 1.4 for SVC and LVC, respectively.

Glucocorticoid treatment drastically increased the E concentration of the superior cervical ganglion of newborn rats (Koslow, Bjegovic and Costa, 1975), but the concentrations of DA and NE were only slightly affected. These effects were interpreted to be due to glucocorticoid induction of the enzyme (phenylethanolamine-N-methyltransferase) responsible for the transformation of NE to E (Ciaranello, Jacobowitz and Axelrod, 1973). The carotid body did not contain any E following glucocorticoid treatment, but the DA and NE concentrations were increased. These changes were observed in carotid bodies from both adult and newborn rats (Koslow and Hellström, unpublished observations). In contrast, those of the superior cervical ganglion only occur in newborn and not in adult rats. In conclusion, glucocorticoids may stimulate the activity in carotid bodies of one or more of the enzymes responsible for the synthesis or degradation of DA and NE. The morphometry results on the granulated vesicles revealed that the storage capacity of the smaller type of vesicles increased relatively more than that of the larger vesicles. This may reflect that the catecholamine which relatively increased the most, NE, is accumulated in the smaller type of vesicles, and that DA is stored in the larger type of vesicles, according to the hypothesis (III) that NE is localized to SVC and DA to LVC.

The physiological significance of the glucocorticoid effects, directly or indirectly mediated, on carotid body CA levels cannot be established at present. But the possibility exists that normally occurring glucocorticoids take part in the regulation of CA levels in carotid body under normal physiological circumstances.
CONCLUSIONS

1. The Type I cells were found to be composed of two subclasses of cells with respect to size and the amount of granulated vesicles. The two groups of cells were named small vesicle cells (SVC) and large vesicle cells (LVC). The LVC and SVC also differed in the volume densities of their nuclei and mitochondria. The mean cell volume of an SVC was about 3/4 of that of an LVC. The majority of nerve-endings terminated on the LVC.

2. Treatment with catecholamine (CA) precursors caused an increased electron-density of the granules of the vesicles. Moreover, the granules almost completely occupied the interior of the vesicles. Similar to the controls, two subclasses of Type I cells existed, namely SVC and LVC. Their granulated vesicles, however, were enlarged and the volume density of the vesicles increased. The changes were most pronounced for the LVC. Many vesicles were located at the periphery of the cells, but without any accumulation close to nerve-endings.

3. The adult carotid body contained large amounts of dopamine (DA) (2.0 nmol/mg protein) and norepinephrine (NE) (1.1 nmol/mg protein). Serotonin was present at a comparatively smaller concentration (0.5 nmol/mg protein). The catecholamine concentration of infant carotid body was lower than that of the adult. The major CA stored in the infant carotid body was similar to that of the adult, DA.

4. Surgical or chemical (6-OH-dopamine) sympathectomy did not change the CA levels of the carotid body. Reserpine depleted the CA content dose-dependently. Treatment with a monoamine oxidase inhibitor increased the DA as well as NE concentrations. L-Dopa manyfolded the amounts of DA and NE; the increase of DA
was most pronounced. Comparing this observation with the increased storage capacities observed in the LVC and SVC (paper II) it is suggested that LVC store DA and that SVC store NE. Inhibition of dopamine-β-hydroxylase reduced the NE concentration without any change in DA level. This supports the hypothesis that DA only partly serves as a precursor of NE and that DA and NE are stored in different cells.

5. After glucocorticoid treatment the NE and DA concentrations of the carotid body increased. The increase of NE was most pronounced. Comparing the storage capacities of the two subclasses of Type I cells revealed that that of SVC increased the most. Thus NE could be localized to SVC and DA to LVC. The effects may reflect a regulatory function of glucocorticoids on carotid body CA levels under physiological conditions.
SAMMANFATTNING AV AVHANDLINGEN


Denna avhandling avsåg att ge en närmare karakteristik av Typ I cellerna och deras granulerade vesiklar och biogena aminer. I studierna användes morfologiska och biokemiska metoder.

I arbete I visas med morfometrisk metodik att Typ I cellerna kan uppdelas i två grupper av celler vad avser dimensionerna hos de granulerade vesiklarna. Den ena gruppen av celler (benämnes små-vesikulära celler/SVC/ ) innehåller vesiklar med snittprofildiametrar omkring 48 nm och den andra gruppen av celler (benämnes stor-vesikulära celler/LVC/ ) innehåller vesiklar med snittprofildiametrar omkring 63 nm. Vid analys av det relativa volymsinnehållet
vesiklar per cell framgår att i LVC upptas dubbelt så stor volym av vesiklar jämfört med i SVC. LVC och SVC skiljer sig också i fråga om volymsinnehåll av mitokond rier och kärna. Jämförelse mellan den totala cellvolymen av en SVC och en LVC visar förhållandet 3:4. De oregel bundna cellutskott som är typiska för Typ I celler upp träder oftare hos LVC än hos SVC. Flertalet av de strukturer som kan identifieras som nervändar ligger i kontakt med LVC. Skillnaderna mellan LVC och SVC, framför allt i storlek och förekomst av granulerade vesiklar, kan av spegla en lagring av olika typer av biogen aminer i LVC och SVC. Möjligheten att iakttagelserna återger olika funktionsstadiator kan dock inte uteslutas.

Inverkan av exogen tillförda katecholaminprekursorer, 5-OH-dopa och L-dopa, på ultrastrukturen hos Typ I cellerna studeras i arbete II. Effekten av 5-OH-dopa-behandling visar sig huvudsakligen relaterad till de granulerade vesiklarna. Granula i vesiklarna ökar i elektronätthet och utfyller dem nästan helt och hållet. Många vesiklar upp träder i cellernas perifera partier nära plasmamembranerna. Det tycks emellertid inte råda någon predilektion för en ansamling av vesiklar i närheten av nervernas kontaktställen. Efter L-dopa-behandling iakttas likartade effekter, dock är elektronättheten i vesikelinnehållet mindre uttalad. Liksom hos normaldjuren föreligger två grupper av Typ I celler med avseende på storlek och förekomst av granulerade vesiklar. Snittprofildiametrarna är emellertid större (för SVC 15 % och för LVC 22 %). Vidare är den relativa volymen vesiklar per cell ökad, mest hos LVC. Fynden talar för att Typ I cellerna kan ta upp prekursorer till katecholaminer och lagra de syntetiserade aminer na i de granulerade vesiklarna. Möjligheter för Typ I cellerna att ackumulera den ökade mängden aminer åstadkoms dels genom att storleken hos vesiklarna ökar, dels genom att mängden vesiklar ökar. Lagringskapaciteten förefaller att öka mest för LVC. Periferställningen av vesiklarna kan möjligen tolkas som ett sätt för vesiklarna att
frigöra sitt innehåll till extracellulärrummet.

I arbete IV studeras effekter av en glukokortikoid (dexamethasone) på Typ I cellernas morfologi och biogena aminer. Mängderna av DA och NE fördubblas. Relativt sett ökar NE mest. E kan inte påvisas alls. Typ I cellerna består, likasom kontrollerna, av två grupper av celler, SVC och LVC. Snittprofilmédeldiametrarna på vesiklarna i SVC såväl som LVC är större än hos kontrollerna (20% resp 10%). Även det relativta volymsinnehållet av vesiklar är ökat, mest för SVC. Det är känt att E-innehållet i t ex ganglion cervicale superius påverkas av exogent tillförda glukokortikoider. Detta anses bero på en inducering av det enzym som överför NE till E. I glomus caroticum iakttas inget E alls, utan i stället ökar mängderna av DA och NE. Detta kan antas bero på en stimulerande effekt av glukokortikoider på någon eller några av de enzymer som deltar i syntesen eller nedbrytningen av NE och DA. Från undersökningen kan man postulera att den amintyp som ökar mest, NE, ackumuleras i den cellgrupp vars lagringskapacitet ökar mest, SVC. Detta är i överensstämmelse med den hypotes som uppställdes i arbete III, d v s att DA huvudsakligen är lokalisert till LVC och NE till SVC. Undersökningen kan vidare tyda på att glukokortikoider under normala fysiologiska betingelser, direkt eller indirekt, deltar i regleringen av CA-innehållet i glomus caroticum.
ACKNOWLEDGEMENTS

I am deeply indebted to Associate Professor Herbert F. Helander, my stimulating teacher, for his unfailing interest and readiness in discussing problems. His generous support and encouragement made it possible for me to complete this study.

My sincere gratitude goes to the late Professor Ebba Cedergren. She introduced me to the field of electron microscopy and evoked my interest in receptor research.

In particular, I would like to thank:

Dr Erminio Costa and my co-author Dr Stephen H. Koslow for their invaluable and generous support and for placing all splendid facilities for the amine-determinations at my disposal,

Docent Tomas Hökfelt for his constructive criticism and valuable advice,

Docent Lars-Eric Thornell and Dr Michael Sjöström, my colleagues at the Department of Anatomy, who have contributed to a stimulating atmosphere, in which I have the privilege to work,

Mr Bror Berggren, Mrs Dollen Bergström and Miss Marja Eirell for their skilful technical assistance,

Mrs Britta Ahlbäck, Mrs Ulla-Britt Granander, Mrs Grethel Jangvad and Miss Leena Jokela for having typed the manuscripts,

all others, whose help, in a variety of ways, has been a prerequisite for the realization of this project.

This investigation was supported by grants from the Medical Faculty, University of Umeå, and the Svenska Sällskapet för Medisinsk Forskning.
REFERENCES


