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Intact and sympathectomized carotid bodies of long-term hypoxic rats: a morphometric ultrastructural study

JEAN-MARC PEQUIGNOT*, STEN HELLSTRÖM and CATHRINE JOHANSSON

Department of Anatomy, University of Umeå, S-901 87 Umeå, Sweden

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Summary

The ultrastructure of the carotid body after exposure to hypoxia (10% O_2) for one, two or three weeks was investigated morphometrically. The study was performed on rats after unilateral removal of the superior cervical ganglion. The normally occurring bimodal distribution of type I cells, representing cells with small vesicle profile diameters (SVC) and large vesicle profile diameters (LVC) respectively, changed after one week of hypoxia into a unimodal population. After one or two weeks of hypoxia the diameter range of dense-cored vesicle (DCV) profiles in type I cells was not different from that of DCV profiles in control LVC. After three weeks of hypoxia the DCV vesicle size was intermediate between those of control SVC and LVC. The volume density of DCV decreased after one week but returned to initial values after two and three weeks of hypoxia. At two or three weeks of hypoxia, however, the total cell volume was increased about 1.4 times which should reflect an increase of the total content of DCV at these times of exposure to hypoxia. An increased mean area of cell profiles indicates a hypertrophy of the type I cells, but no signs of hyperplasia could be detected. The ganglionectomy did not cause any remarkable changes compared to the intact carotid body except for a higher volume density of DCV during the early periods of hypoxia.

It is inferred from the study that the increased total mass of type I cell tissue during long-term hypoxia is due to a hypertrophy of the cells. Furthermore, the type I cells can increase their storage capacity for catecholamines during hypoxia by an increase in the size and number of DCV.

Introduction

The enlarged carotid bodies of animals exposed to long-term hypoxia show several morphological alterations, including a pronounced vasodilatation and an increase in the amounts of both glomus cells and connective tissue (Edwards *et al.*, 1971; Blessing &

**Present address*: ER CNRS 196, Laboratoire de Physiologie, Faculté de Médecine Grange-Blanche, 69373 Lyon Cédex 8, France.

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Wolff, 1973; Laidler & Kay, 1975). Several questions, however, remain to be answered conclusively, e.g. does the increased total content of type I cells represent hypertrophy and/or hyperplasia?

Although the effects of chronic hypoxia on the ultrastructure of type I cells have been extensively examined, the studies have yielded different results regarding the effects on the dense-cored vesicles (DCV) (Møller *et al.*, 1974; Blessing & Kaldeweide, 1975; Laidler & Kay, 1978). These conflicting results could depend on differences in the conditions of hypoxia and certainly on species differences (Alfes *et al.*, 1977; Fidone *et al.*, 1980). There is evidence that DCV contain catecholamines (CA), mainly dopamine and to a lesser extent norepinephrine (for review, see Verna, 1979). Recently it was shown that the CA content in the carotid body was gradually enhanced in rats breathing 10% O_2 in nitrogen for one month (Hanbauer *et al.*, 1981). After four weeks of hypoxia the content of dopamine was increased 15-fold and that of norepinephrine was increased 12-fold. These effects elicited by long-term hypoxia were augmented after removal of the sympathetic superior cervical ganglia. Thus the question arises whether these biochemical alterations are reflected in the carotid body morphology by an increase in size and/or number of the DCV, thus providing an increased storage capacity for CA.

Using stereological methods, we have investigated the effects of long-term hypoxia on the ultrastructural features of type I cells in intact and sympathectomized carotid bodies of rats. The electron microscopical examinations were performed at one, two or three weeks of exposure to hypoxia. Particular attention was paid to DCV in attempts to correlate these morphometric data with the earlier quantitative results on CA during similar hypoxic conditions.

Materials and methods

Twenty-five male Sprague–Dawley rats (150–200 g) were used for the investigation. The animals were the same as used in the light microscopical study previously published (Pequignot & Hellström, 1983), in which the experimental procedure is described in detail.

Sympathectomy

The left superior cervical ganglion was removed surgically. The animals were allowed to recuperate for seven days before being killed (ganglionectomized controls, $n = 5$) or exposure to long-term hypoxia (hypoxic rats, $n = 15$).

Long-term exposure to hypoxia

Three groups of five unilaterally ganglionectomized rats each were kept in a normobaric hypoxic chamber (10 ± 0.5% O_2 in N_2), for one, two and three weeks respectively. The CO_2 concentration inside the chamber was 0.88 ± 0.05%.

Intact controls

Five untreated rats were exposed only to room air and used as intact controls.

Histological studies

At the time intervals described above, the hypoxic rats, while still in the hypoxic chamber, were anaesthetized with intraperitoneal pentobarbital sodium. Then the rats were rapidly connected to a perfusion apparatus and perfused through the left cardiac ventricle for 10 min with the fixative (2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.40; flow rate 20 ml min^{-1}, pressure 120 cm H_2O). The carotid bodies were dissected free and further fixed by immersion for 2 h in the same solution. The tissues were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer, dehydrated in increasing concentrations of ethanol and embedded in Epon. Ultrathin sections were analysed at each of four different levels in every carotid body. The interval between two consecutive sections was 25 μm for control and 50 μm for one-, two- and three-week hypoxic carotid bodies. From each level, 7–10 type I cells (in all about 30 cells from each carotid body), selected at random, were photographed. Only cells which displayed their nucleus in the section were studied.

Calibration of the magnification was performed using a carbon grating with 2160 lines mm^{-1}. The mean cell area and the volume densities of DCV, mitochondria and nuclei were estimated by a point-counting method (Weibel, 1969) on prints with a magnification of about × 15 000. The diameters of DCV profiles were measured with a Zeiss Particle Analyzer TGZ-3 on prints with a final magnification of about × 30 000. On semithin section (1 μm), cut at the same levels as the ultrathin sections, the number of type I and type II cell nuclei were counted at a total magnification of × 1200 times in a light microscope under an oil-immersion objective.

Statistical evaluation of the data was made by Student's *t*-test. Differences in distributions of DCV median diameters were tested by using the Kolmogorov–Smirnov (two-tailed) two sample test (Siegel, 1956). In this test a P value is calculated from the number of observations and the maximal difference in the cumulative frequency distribution (D_{max}). Differences were considered to be significant if $P < 0.05$.

Results

Intact and sympathectomized carotid bodies of control rats

Measuring the diameters of DCV profiles revealed two populations of type I cells, which correspond to the small vesicle cells (SVC) and large vesicle cells (LVC) described earlier (Hellström, 1975; and type B and type A respectively according to McDonald & Mitchell, 1975) (Fig. 1). The size of the vesicle profiles ranged from 30 to 85 nm and from 30 to 125 nm in SVC and LVC respectively. Once distinguished by the diameters of their DCV (Fig. 3), SVC and LVC were found to have other differing characteristics as previously reported (Hellström, 1975; McDonald & Mitchell, 1975). The mean cell area of the LVC was larger than that of the SVC and the volume densities of DCV and mitochondria were higher in LVC compared to SVC (Table 1).

Similarly to the intact controls, two populations of type I cells were also found in the ganglionectomized carotid bodies. The quantitative data of their ultrastructural features did not differ from those of intact controls (Table 1).

Intact and sympathectomized carotid bodies of hypoxic rats

Fig. 2 shows the ultrastructural appearance of some type I cells of a carotid body from a hypoxic rat. DCV of carotid bodies from the hypoxic rats exhibited the same ultrastructural features as the controls, i.e. a membrane-bound granule surrounded by

the clear halo. Only occasionally did the dense core appear eccentric and adherent to the vesicle membrane.

Measurement of the diameter of vesicle profiles revealed a unimodal distribution of type I cells during the various times of hypoxia (Fig. 3). This alteration of type I cells from a bimodal to a unimodal distribution with respect to the size of their DCV was obvious already after one week of hypoxia. At each step of hypoxia, the median diameter range of DCV profiles was greater than that of DCV profiles in control SVC (D_{max} = 35.5%, 44.2% and 44.0% after one, two and three weeks of hypoxia respectively, $P < 0.001$ for each value). On the other hand there was no significant difference between the size range of DCV after one or two weeks of hypoxia and that of DCV in control LVC (D_{max} = 16.3% and 14.3% after one and two weeks of hypoxia respectively, $P < 0.10$ for each value). However, after three weeks of hypoxia the DCV size was slightly smaller than that observed in control LVC (D_{max} = 20.6%, $P < 0.05$) (Table 1, Fig. 3).

Comparing the volume density of DCV of the type I cells from the hypoxic animals with that of LVC in controls showed DCV to decrease after one week but to return to initial values after two and three weeks of hypoxia (Table 1). Comparing the DCV data with that of control SVC however showed an increase after two and three weeks of hypoxia. There was no significant alteration in the volume density of mitochondria (Table 1).

The mean cell area seemed to increase considerably, already about 30% after one week of hypoxia (Table 1). The volume density of the nuclei was decreased, thus indicating the major change in cell area to be due to an enlargement of the cytoplasmic volume.

Assuming the cells to be spheres and that the cells analysed (displaying their nucleus) were sectioned close to their centres, the cell volume can be roughly estimated from the mean cell profile area. When compared with LVC controls, the volume of type I cells was increased about 1.4 times after two or three weeks of hypoxia.

Ganglionectomy did not significantly affect the ultrastructural alterations elicited by long-term hypoxia, although the volume density and content of DCV in type I cells of ganglionectomized carotid bodies were slightly higher at one week of hypoxia compared with the intact side ($P > 0.05$, Table 1). The median diameter distributions of the vesicle profiles were not different from those of DCV profiles in type I cells of intact carotid bodies in normoxic (D_{max} = 4.2%, $P > 0.10$) and hypoxic rats (D_{max} = 18.3%, 9.8% and 11.9% after one, two and three weeks of hypoxia, $P < 0.10$ for each value). However, the cluster analysis of the DCV median diameters revealed that the conversion from a bimodal to a unimodal distribution seemed to occur faster in the sympathectomized carotid bodies (Fig. 3).

Fig. 1. Electron micrograph showing type I and type II cells of an intact carotid body from a control rat. The cytoplasm of type I cells contains dense-cored vesicles. Two small vesicle cells (SVC) and three large vesicle cells (LVC) are indicated. × 10 000.

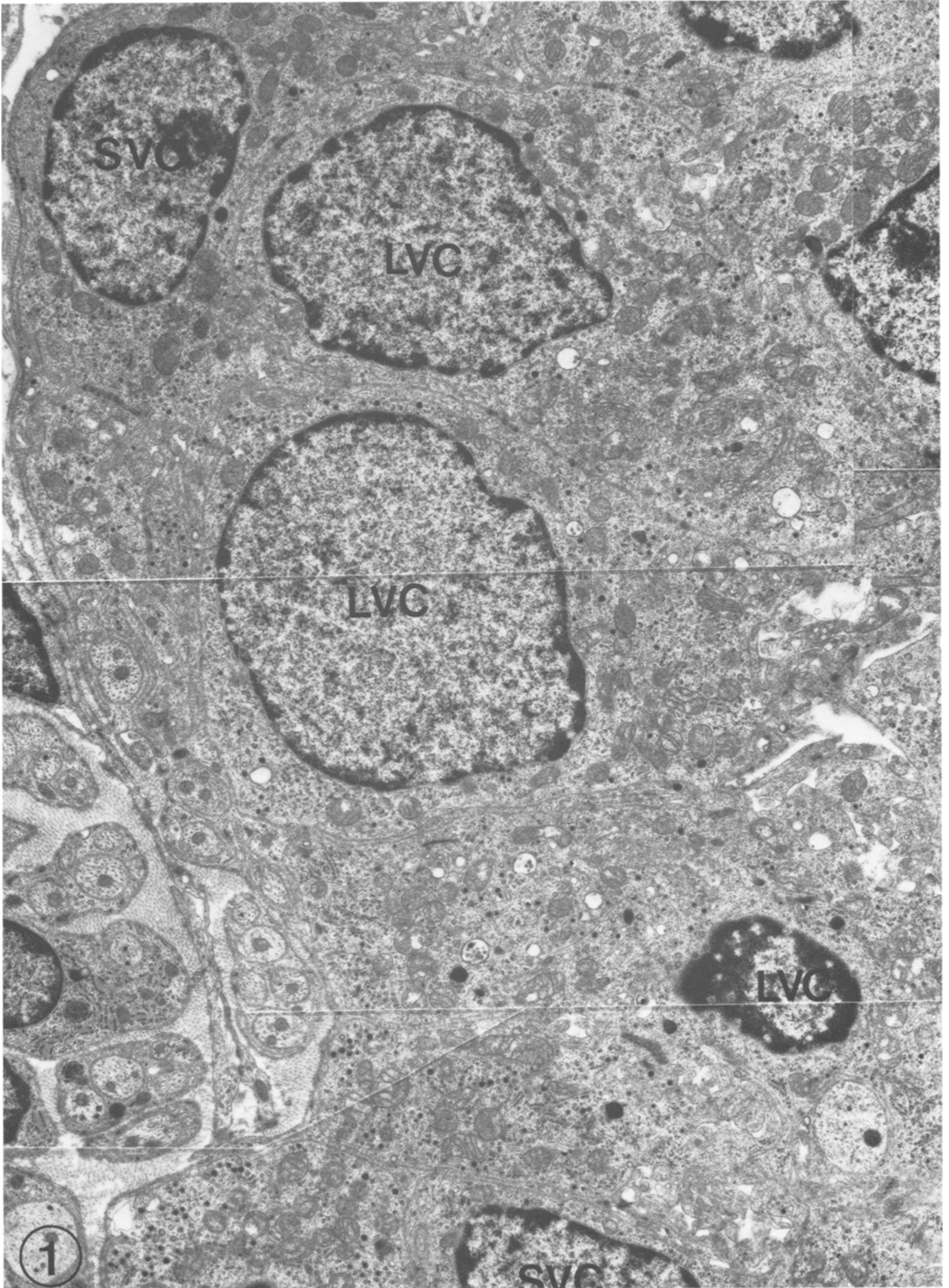
SVC
LVC
LVC
LVC
SVC
1

Table 1. Quantitative morphological data of intact and sympathectomized carotid bodies after different intervals of exposure to hypoxia.

Duration of hypoxia	*Number of cells*	*Mean diameter of vesicle profiles* (nm)	*Mean cell area* (μm^2)	*Nucleus volume density* (%)	*Mitochondrial volume density* (%)	*Volume density of DCV* (%)
Control						
Intact						
Small vesicle cells (SVC)*	74	54.0 ± 0.5	43.3 ± 3.9 (5)	51.3 ± 1.8 (5)	12.9 ± 1.1 (5)	1.40 ± 0.09 (5)
Large vesicle cells (LVC)†	94	73.6 ± 0.7	54.7 ± 2.7 (5)	42.7 ± 1.8 (5)	16.0 ± 0.4 (5)	2.21 ± 0.17 (5)
Ganglionectomy						
SVC	73	53.4 ± 0.5	48.8 ± 4.0 (4)	48.1 ± 2.2 (4)	13.0 ± 1.0 (4)	1.32 ± 0.11 (4)
LVC	49	73.4 ± 1.1	57.8 ± 2.8 (4)	46.5 ± 2.4 (4)	14.1 ± 0.8§ (4)	2.24 ± 0.11 (4)
Hypoxia 1 week						
Intact	138	69.7 ± 1.0	65.1 ± 3.3‡ (5)	41.1 ± 1.5 (5)	13.6 ± 0.4‡ (5)	1.23 ± 0.10‡ (5)
Ganglionectomy	139	70.5 ± 0.8	63.3 ± 1.9 (5)	38.9 ± 0.7‡ (5)	15.1 ± 0.8 (5)	1.57 ± 0.09‡§ (5)
Hypoxia 2 weeks						
Intact	112	72.1 ± 0.7	67.6 ± 4.2‡ (4)	37.9 ± 2.5‡ (4)	14.4 ± 0.3‡ (4)	1.92 ± 0.15 (4)
Ganglionectomy	142	71.1 ± 0.7	68.7 ± 4.0‡ (5)	37.9 ± 0.6‡ (5)	15.1 ± 0.8 (5)	2.08 ± 0.29 (5)
Hypoxia 3 weeks						
Intact	144	67.8 ± 0.6	69.4 ± 0.9‡ (5)	36.3 ± 0.8‡ (5)	15.2 ± 0.7 (5)	2.36 ± 0.23 (5)
Ganglionectomy	145	68.5 ± 0.7	64.3 ± 2.1‡§ (5)	37.4 ± 1.2‡ (5)	14.9 ± 0.9 (5)	2.11 ± 0.23 (5)

*Corresponds to the type B cell (McDonald & Mitchell, 1975).
†Corresponds to the type A cell (McDonald & Mitchell, 1975).
‡$P < 0.05$ hypoxia versus normoxia.
§$P < 0.05$ sympathectomized carotid bodies versus intact carotid bodies.
Values expressed as mean ± S.E.M.; number of animals within parentheses.

Determination of total counts of type I and type II cell nuclei

In an attempt to compare the total number of type I and type II cells the number of nuclei of these cells was estimated at four different levels from the main portion of the carotid body. This rough estimate of the total number of type I cell and type II cell nuclei of the carotid bodies from hypoxic animals showed no differences from intact or ganglionectomized controls (Table 2). Type I cells exhibiting mitotic figures were not identified in any of the type I cells analysed (about 34 000).

Discussion

The present study showed that the increase in absolute volume of type I cells in the enlarged carotid body during chronic hypoxia seems to be due to a hypertrophy and not to a hyperplasia. Long-term hypoxia elicited a hypertrophy of the type I cells within one week. Using quantitative methods, Laidler & Kay (1978) reported a three-fold increase in volume of the type I cells in rats exposed to a simulated altitude of 4300 m for four or five weeks. Although the hypoxic rats in our study were submitted to a higher stimulus (equivalent to 5500 m) the hypertrophy of type I cells was less developed and was not further increased when the period of hypoxia was extended to three weeks.

As recently reported (Pequignot & Hellström, 1983), the time-course of structural alterations of type I cells, i.e. the volume of DCV correlated to the hypertrophic cells, seems to be unable to account for the observed gradual increase in CA content of rat carotid body, under long-term hypoxia (Hanbauer *et al.*, 1981). At an ultrastructural level, Møller *et al.* (1974) reported an increase in the number of DCV in type I cells of rabbits living at an altitude of 4000–4300 m or exposed to a simulated altitude of 6000 m for one week. On the contrary, Blessing & Kaldeweide (1975) found a decrease in the number of DCV in rats progressively adapted to a simulated altitude of 7000 m. Such varying results could be derived from the marked species difference in CA content of the carotid body (Alfes *et al.*, 1977; Fidone *et al.*, 1980).

The only study, however, in which stereological measurements have been used, is that by Laidler & Kay (1978). In the carotid bodies of their long-term hypoxic rats, these authors found an increased diameter of DCV. They also reported a two-fold decrease in the number of DCV per μm^2 of type I cell cytoplasm and a small decrease or no change in the number of vesicles per type I cell profile. The present study showed that the type I cells could increase their CA storage capacity at least in part by two successive mechanisms:

1. Already after one week of hypoxia, the normally occurring bimodal distribution of type I cells (Hellström, 1975; McDonald & Mitchell, 1975) was transformed into a unimodal distribution. At each step of hypoxia, the DCV in type I cells were larger than those in SVC from controls. Moreover, at least until two weeks of hypoxia the size range of DCV was not significantly different from that of DCV in control LVC. When considering the ratio of LVC and SVC in controls (LVC:SVC = 1.27), the increased

diameters of DCV (about 7 and 11% after one and two weeks of hypoxia respectively) will increase the volumes of the vesicles by a factor of approximately 1.2 and 1.4 respectively. The size range of DCV became slighly smaller than that of vesicles in control LVC after three weeks of hypoxia: thus the net increase in the volume of DCV will be less pronounced at this point of time, corresponding to a factor of about 1.2. Unlike the present study, Laidler & Kay (1978) found a bimodal distribution of the mean diameters of vesicles in type I cells of hypoxic as well as normoxic rats.

2. Relating the volume density of DCV to the hypertrophy of the type I cells, there was a net increase in content of DCV within two and three weeks of hypoxia. The changes of volume densities of DCV in response to long-term exposure to hypoxia were due to an increase in both size and number of vesicles. The DCV enlargement however only contributed to a minor part of the alterations in DCV volume densities, namely 13, 19 and 8% after one, two and three weeks of hypoxia respectively. Taking these moderate changes into account, it could be calculated that the number of vesicles per type I cell profile increased about 1.3-fold and 1.9-fold after two and three weeks of hypoxia respectively. Concomitant with the changes in DCV volume density there was an increased amount of mitochondria, suggesting an augmented metabolic activity in the type I cells.

The combined enlargement of DCV and their increased number enhanced total volume of DCV approximately two-fold, a low value compared to the reported 6–10-fold increase of CA content of the long-term hypoxic carotid bodies (Hanbauer *et al.*, 1981). Because a significant extravesicular CA storage in type I cells is unlikely, it can be inferred that the CA storage capacity of type I cells was enhanced by increasing the CA concentration in each granule rather than the total volume of DCV.

The physiological significance of the two different populations of type I cells in carotid body of normoxic rat (Hellström, 1975; McDonald & Mitchell, 1975) is still debated. It has been proposed that these cells store different kinds of neurochemicals. In a recent immunocytochemical study performed on carotid bodies of rat and cat, Chen & Yates (1981) localized the dopamine-β-hydroxylase in many large DCV but not in the small vesicles. They concluded that the LVC are noradrenergic rather than dopaminergic. Other immunohistochemical studies in rat (Bolme *et al.*, 1977), bat and dog (Karasawa *et al.*, 1982) support the view that the type I cells are exclusively dopaminergic in these species. On the other hand, if norepinephrine and dopamine are stored in different types of vesicles, it is difficult to explain the opposite changes in size of both vesicle populations whereas the content of both CAs was increased (Hanbauer *et al.*, 1981). If

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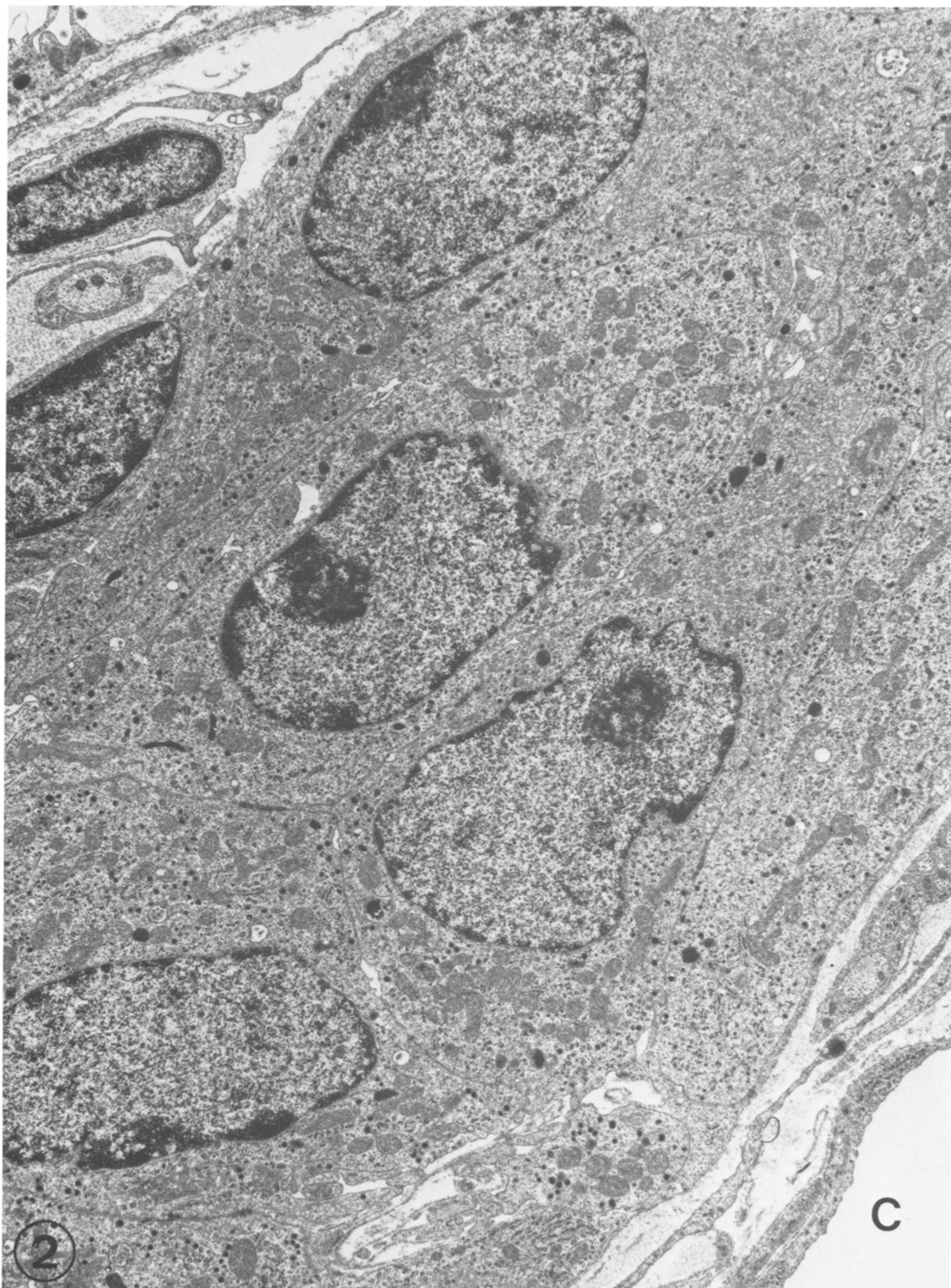
Fig. 2. Electron micrograph showing type I cells of an intact carotid body from a rat exposed to three weeks of hypoxia. The cytoplasm is enlarged. New blood vessels develop inside the glomus islands. Note the close connection between a capillary (C) and the cluster of type I cells. × 10 000.


C
2

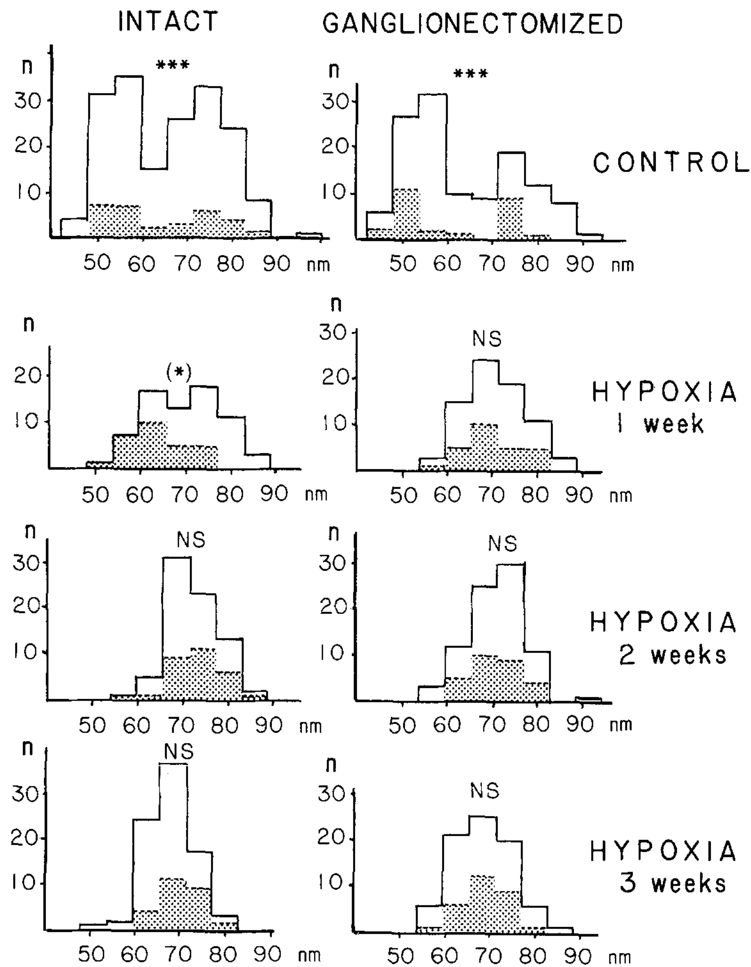


Fig. 3. Histograms of the median diameters of the vesicle profiles of individual type I cells in each experimental group. The dotted histograms represent about 30 cells from one carotid body in each group. The data have been tested by cluster analysis (Engelman & Hartigan, 1969) with respect to the probability that two groups of cells exist. The test was performed by a computer. Note that there is still a tendency of a bimodal population in intact carotid bodies after one week of hypoxia. At the same time interval the unimodal distribution has been fully achieved in sympathectomized carotid bodies. n, number of cells; ***, $P < 0.01$; (*), $0.05 < P < 0.10$; NS, not significant.

different neurochemicals are not stored, the two populations of type I cells could represent two different functional conditions, e.g. resting or active cells with synthesis and storage plus release functions, respectively, as has been proposed for the two populations of granules in sympathetic nerves (Fried *et al.*, 1983). Within three weeks of hypoxia, type I cells, with DCV of an intermediate size, seemed to develop. This phenomenon may have several explanations. Either SVC and LVC were both

Table 2. Total number of type I and type II cells counted at four different levels of each carotid body.

	Number of nuclei	
Duration of hypoxia	*Type I cells*	*Type II cells*
Control		
Intact	874 ± 99 (5)	91 ± 16 (5)
Ganglionectomized	730 ± 131 (5)	66 ± 64 (5)
Hypoxia 1 week		
Intact	725 ± 75 (5)	61 ± 9 (5)
Ganglionectomized	719 ± 108 (5)	66 ± 7 (5)
Hypoxia 2 weeks		
Intact	938 ± 64 (5)	80 ± 12 (5)
Ganglionectomized	968 ± 77 (4)	73 ± 15 (4)
Hypoxia 3 weeks		
Intact	760 ± 130 (5)	66 ± 14 (5)
Ganglionectomized	615 ± 79 (5)	72 ± 6 (5)

Values expressed as mean ± S.E.M.; number of animals analysed within parentheses.

transformed into a third type of glomus cell or both cell types underwent changes in vesicle morphology but still existed as separate cell types. On the basis of the median vesicle profile diameter, estimated for each cell, the cluster analysis (Engelman & Hartigan, 1969) was used to separate LVC from SVC. This test attempts to find the point in a distribution with the highest probability that it could be divided into two groups. At this point the distribution will be sharply cut in two halves not taking into account that each of these halves may be tailing into each other. In the present study it is possible that the cluster analysis, at the point of division, characterized some of the SVC containing somewhat larger median vesicle profile diameters as LVC and some LVC with somewhat smaller median vesicle profile diameters as SVC. This will affect the mean values of the vesicle profile diameters in such a way that e.g. the diameters of vesicle profiles of control LVC will become slightly too large. Against this background it is difficult to judge whether the mean vesicle profile diameter of control LVC really differs from that estimated for the hypoxic carotid bodies or not.

Little is known about the mechanism whereby sympathetic nervous activity regulates CA metabolism during long-term hypoxia. Since the major part of sympathetic neurons innervating the carotid body terminate on blood vessels, we previously hypothesized that sympathetic nerve activity could operate by reducing the local blood flow. Evidence against this hypothesis is the fact that sympathectomy did not change the luminal size of the blood vessels of rat carotid body during long-term hypoxia (Pequignot & Hellström, 1983). Another site of regulatory activity from sympathetic nerve endings could be the walls of blood vessels as suggested by studies on pulmonary arteries in

long-term hypoxic animals (Naeye, 1965; Meyrick & Reid, 1978). Yet it is unknown whether chronic stimulation of sympathetic nerves can produce changes in ultrastructure of the vessel walls within the carotid body. Recent electrophysiological investigations also indicate the existence of nonvascular mechanisms of modulation of chemoreceptor activity by autonomic nerves (Acker & O'Regan, 1981; O'Regan, 1981). Histological studies have demonstrated the presence of pre- and postganglionic cervical sympathetic endings in close proximity to the type I cells whose activity could be thus influenced by such a nerve supply (see Verna, 1979, and McDonald, 1981).

Although sympathectomy leads to a net increase in CA stores in carotid bodies of rats exposed to two weeks of hypoxia (Hanbauer *et al.*, 1981), the ultrastructural changes elicited by ganglionectomy in hypoxic type I cells were less obvious and were related to exposure time in hypoxic atmosphere. The volume density and amount of DCV were augmented by sympathectomy in rats exposed to one week of hypoxia. The difference in DCV amounts between ganglionectomized and intact rats was less after two weeks ($P < 0.10$) and disappeared after three weeks of hypoxia. Although sympathectomy did not affect the size range of DCV, the development of a unimodal distribution of type I cells under long-term hypoxia seemed to occur faster in the sympathectomized carotid bodies (Fig. 3). Thus, it seems that sympathectomy speeded up the ultrastructural alterations elicited by long-term hypoxia. To clarify this point, it would be interesting to investigate the time-course of CA changes induced by sympathectomy in carotid bodies of long-term hypoxic rats.

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References

ACKER, H. & O'REGAN, R. G. (1981) The effects of stimulation of autonomic nerves on carotid body blood flow in the cat. *Journal of Physiology* **325**, 99–110.

ALFES, H., KINDLER, J., KNOCHE, H., MATTHIESSEN, D., MOLLMANN, H. & PAGNUCCO, R. (1977) The biogenic amines in the carotid body. *Progress in Histochemistry and Cytochemistry* **10**, 1–69.

BLESSING, M. H. & KALDEWEIDE, J. (1975) Light and electron microscopic observations on the carotid bodies of rats following adaptation to high altitude. *Virchows Archiv Abteile B, Cell Pathology* **18**, 315–29.

BLESSING, M. H. & WOLFF, H. (1973) Befunde am Glomus caroticum der Ratte nach Aufenthalt in einer simulierten Höhe von 7500 m. *Virchows Archiv Abteile A, Pathological Anatomy* **360**, 79–92.

BOLME, P., FUXE, K., HÖKFELT, T. & GOLDSTEIN, M. (1977) Studies on the role of dopamine in cardiovascular and respiratory control: central versus peripheral mechanisms. *Advances in Biochemical Psychopharmacology* **16**, 281–90.

CHEN, I. L. & YATES, R. D. (1981) Immunocytochemical localization of dopamine β-hydroxylase (DBH) in the carotid body. *Journal of Cell Biology* **91**, 88a.

EDWARDS, C., HEATH, D., HARRIS, P., CASTILLO, Y., KRÜGER, H. & ARIAS-STELLA, J. (1971) The carotid body in animals at high altitude. *Journal of Physiology* **104**, 231–8.

ENGELMAN, L. & HARTIGAN, J. A. (1969) Percentage points of a test for clusters. *Journal of the American Statistical Association* **64**, 1647–8.

FIDONE, S. J., GONZALEZ, C. & YOSHIZAKI, K. (1980) Putative neurotransmitters in the carotid body: the case for dopamine. *Federation Proceedings* **39**, 2636–40.

FRIED, G., LAGERCRANTZ, H., KLEIN, R. L. & THURESON-KLEIN, Å. (1983) Large and small vesicles. Origin, contents and functional significance. 5th CA Symposium Göteborg 1983. *Progress in Neuropsychopharmacology and Biological Psychiatry*, Suppl. 3–4.

HANBAUER, I., KAROUM, F., HELLSTRÖM, S. & LAHIRI, S. (1981) Effects of hypoxia lasting up to one month on the catecholamine content in rat carotid body. *Neuroscience* **6**, 81–6.

HELLSTRÖM, S. (1975) Morphometric studies of dense-cored vesicles in type I cells of rat carotid body. *Journal of Neurocytology* **4**, 77–86.

KARASAWA, N., KONDO, Y. & NAGATSU, I. (1982) Immunohistochemical and immunofluorescent localization of catecholamine-synthesizing enzymes in the carotid body of the bat and dog. *Archivum histologicum japonicum* **45**, 429–35.

LAIDLER, P. & KAY, J. M. (1975) A quantitative morphological study of the carotid bodies of rats living at a simulated altitude of 4300 m. *Journal of Physiology* **117**, 183–91.

LAIDLER, P. & KAY, J. M. (1978) A quantitative study of some ultrastructural features of the type I cells in the carotid bodies of rats living at a simulated altitude of 4300 metres. *Journal of Neurocytology* **7**, 183–92.

McDONALD, D. M. (1981) Peripheral chemoreceptors. Structure–function relationships of the carotid body. In *Regulation of Breathing* (edited by HORNBEIN, T. F.), pp. 105–319. New York: Marcel Dekker.

McDONALD, D. M. & MITCHELL, R. A. (1975) The innervation of glomus cells, ganglion cells and blood vessels in rat carotid body: a quantitative ultrastructural analysis. *Journal of Neurocytology* **4**, 177–230.

MEYRICK, B. & REID, L. (1978) The effect of continued hypoxia on rat pulmonary arterial circulation. An ultrastructural study. *Laboratory Investigation* **38**, 188–200.

MØLLER, M., MØLLGARD, K. & SØRENSEN, S. C. (1974) The ultrastructure of the carotid body in chronically hypoxic rabbits. *Journal of Physiology* **238**, 447–53.

NAEYE, R. L. (1965) Effect of α-methyl-dopa on heart and pulmonary arteries of hypoxic mice. *American Journal of Physiology* **209**, 702–4.

O'REGAN, R. G. (1981) Responses of carotid body chemosensory activity and blood flow to stimulation of sympathetic nerves in the cat. *Journal of Physiology* **315**, 81–98.

PEQUIGNOT, J. M. & HELLSTRÖM, S. (1983) Intact and sympathectomized carotid bodies of long-term hypoxic rats. A morphometric light microscopical study. *Virchows Archives Abteile A, Pathological Anatomy and Histology* **400**, 235–43.

SIEGEL, S. (1956) *Nonparametric Statistics for the Behavorial Sciences*, pp. 127–31. New York: McGraw-Hill.

VERNA, A. (1979) Ultrastructure of the carotid body in the mammals. *International Review of Cytology* **60**, 271–330.

WEIBEL, E. R. (1969) Stereological principles for morphometry in electron microscopic cytology. *International Review of Cytology* **26**, 235–302.