Virchows Arch (Pathol Anat) (1983) 400:235–243

Virchows Archiv A

Intact and sympathectomized carotid bodies of long-term hypoxic rats

A morphometric light microscopical study

Jean-Marc Pequignot*, and Sten Hellström
Department of Anatomy, University of Umeå, S-901-87 Umeå, Sweden

Summary. Intact and sympathectomized carotid bodies from rats exposed to hypoxia (room-air mixed with N_2 to a final oxygen concentration of 10%) for 1, 2 and 3 weeks were analyzed by morphometric methods using the light microscope. Exposure to hypoxia enlarges the carotid bodies, in which the vascularity increases dramatically from the first week. This changed vascularity seems to be due to enhanced vasodilatation. In addition, there is a pronounced profileration of the endothelial tissue, thus suggesting an ingrowth of new blood vessels. The volume density of glomic type I, and type II cells, decreases during hypoxia but when calculated in total volumes this entity is increased 3-fold. Sympathectomy does not modify the structural changes occurring during hypoxia when compared with carotid bodies with intact innervation. Though the primary elements in the mechanism of transduction in carotid body are unknown, a remodelling of the vascular architecture may be a method by which this chemoreceptor alters its sensitivity

Key words: Carotid body – Long-term hypoxia – Sympathectomy – Morphometry

The peripheral arterial chemoreceptors are sensitive to hypoxia. Long-term hypoxia, induced by high altitude or simulated height, elicits a number of structural changes in the carotid body. One of the most obvious alterations is a general enlargement of the organ, which has been observed in man as well as several animal species including dog, rabbit, guinea-pig and rat (Arias-Stella 1969; Barer et al. 1972; Blessing and Wolff 1973; Edwards 1971). Light microscopical studies have emphasized that this enlargement is mainly due to a dilatation of capillaries and to a lesser degree to an

* *Offprint requests to:* J.-M. Pequignot's present address: ER CNRS 196, Laboratoire de Physiologie; Faculté de Médecine Grange-Blanche 8, avenue Rockefeller, F-69373 Lyon Cédex 08, France

increase in the total volume occupied by glomic cells as well as connective tissue (Blessing and Wolff 1973; Laidler and Kay 1975a). However, the dynamics of the structural changes induced by long-term hypoxia have not been defined.

From a biochemical point of view, the type I cells of carotid body contain catecholamines, mainly dopamine but also noradrenaline (Hellström and Koslow 1975). Recently, Hanbauer et al. (1981) reported an increased dopamine and noradrenaline content in carotid bodies of rats exposed to 10% O_2 in nitrogen for 1 week. Prolonged hypoxia for 2 and 4 weeks, elicited a further increase in the amount of catecholamines. It was also shown that these effects on catecholamines by long-term hypoxia were enhanced in sympathectomized carotid bodies. The mechanism whereby sympathetic nerves can regulate the catecholamine metabolism in carotid body is unknown, however. From a classical point of view the sympathetic nerve stimulation can alter the carotid body activity by decreasing the local blood flow (Belmonte and Eyzaguirre 1974; Purves 1970).

In the present quantitative light microscopical study we investigated the changes elicited by long-term hypoxia in intact and sympathectomized rat carotid bodies. The examinations were performed at 3 different time intervals of exposure to normobaric 10% hypoxia.

Material and methods

Twenty-five male Sprague-Dawley rats (150–200 g) were used for investigation. All animals, 1 month old, were kept in the same climatized room at sea level, at 26° C, food and water *ad* libitum and with a 12 h light-dark cycle.

Sympathectomy. Twenty male rats were anaesthetized with hexobarbital sodium through a tail vein and the left superior cervical ganglion removed. The animals were allowed to recuperate for 7 days before killing (ganglionectomized controls, $n=5$) or exposure to chronic hypoxia (hypoxic rats, $n=15$).

Long-term exposure to hypoxia Three cages containing 5 ganglionectomized rats each were put into a chamber supplied with a flow of air and 100% nitrogen, adjusted to a final O_2 concentration of $10\pm0.5\%$ inside the chamber. The control of CO_2 was achieved by soda lime inside the hypoxic chamber. The O_2 and CO_2 concentrations were monitored, twice daily, on Beckman analyzers. The three groups of rats stayed in the hypoxia atmosphere for 1, 2 and 3 weeks respectively. Though the arterial levels of blood gases were not determined, it can be inferred from previous observations (Lewis et al., 1974) that paO_2 falls close to 40 mm Hg in less than 1 h in rat breathing an air-nitrogen mix at 10% O_2.

Intact controls. Five non-ganglionectomized rats were exposed to room air only and used as intact controls.

Histological studies. Each hypoxic and control rat was anaesthetized with intraperitoneal pentobarbital sodium. The hypoxic rats were kept in the hypoxic chamber until the anaesthesia was performed. Then the rats were immediately perfused through the left heart ventricle for 10 min with a fixative (2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.40; flow rate 20 $ml \cdot min^{-1}$). The time between the anaesthetized rat leaving the cage and the perfusate entering the aorta was less than 2 min. The carotid bodies were dissected free of connective tissue and processed further for microscopy. The tissues were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 2 h, dehydrated in increasing concentrations of ethanol and embedded

in Epon 812. Semithin (0.5 μm) sections were analysed at each of 4 different levels in every carotid body. The interval between two consecutive sections was 25 μm for control and 50 μm for 1-, 2- and 3 week hypoxic carotid bodies.

The volume proportions of 4 tissue constituents within the carotid body were estimated by point-counting according to Weibel (1969). The tissue constituents counted were: glomic cells (the islands containing type I and type II cells), blood vessel lumen, endothelial cells and interstitial tissue.

Data were analysed using Student's *t*-test and differences considered statistically significant when $p < 0.05$.

Results

The intact and sympathectomized carotid bodies of control rats

The glomic cells of intact carotid bodies are arranged in compact clusters separated each from one another by numerous blood vessels and connective tissue (Fig. 1 a). Left and right carotid bodies are similar in appearance. Sympathectomy does not seem to alter the general morphology (Fig. 1 b). The connective tissue seems to be more abundant yielding, in some specimens, a looser structural organization. One sympathectomized carotid body differs completely: it exhibits only some small glomic islands and blood vessels scattered in a large amount of connective tissue and was therefore discarded from this study.

Quantitative measurements confirm that the left and right intact carotid bodies do not differ with respect to the main tissue components (Table 1). Sympathectomy increases the volume density of interstitial tissue but fails to change the volume of other tissue constituents.

The intact and sympathectomized carotid bodies of hypoxic rats

In intact as well as sympathectomized specimens, the most striking feature of the hypoxic carotid bodies is their tremendously increased vascularity which is already evident after 1 week of exposure to hypoxia (Fig. 1 c–h). The capillaries are dilated and in many intances seem to be located within the glomic islands.

The quantitative data are shown in Table 1. When compared to intact controls, the volume density of the blood vessel lumen is increased from the first week of hypoxia and is further augmented within 2 weeks. A prolonged hypoxic exposure for 3 weeks does not further increase the volume density of blood vessel lumen. The volume density of endothelial cells remains almost constant regardless of exposure time to hypoxia. The same pattern is noted for interstitial tissue, whereas the volume density of glomic cells is slightly decreased.

The sympathectomized carotid bodies exhibit the same structural changes as intact carotid bodies (Table 1) during long-term hypoxia except that the volume density of endothelial cells is significantly increased in the sympathectomized carotid bodies.

The changes in amount of each tissue constituent during hypoxia were assessed using Simpson's rule (Dunnill 1967). The Fig. 2 shows that the

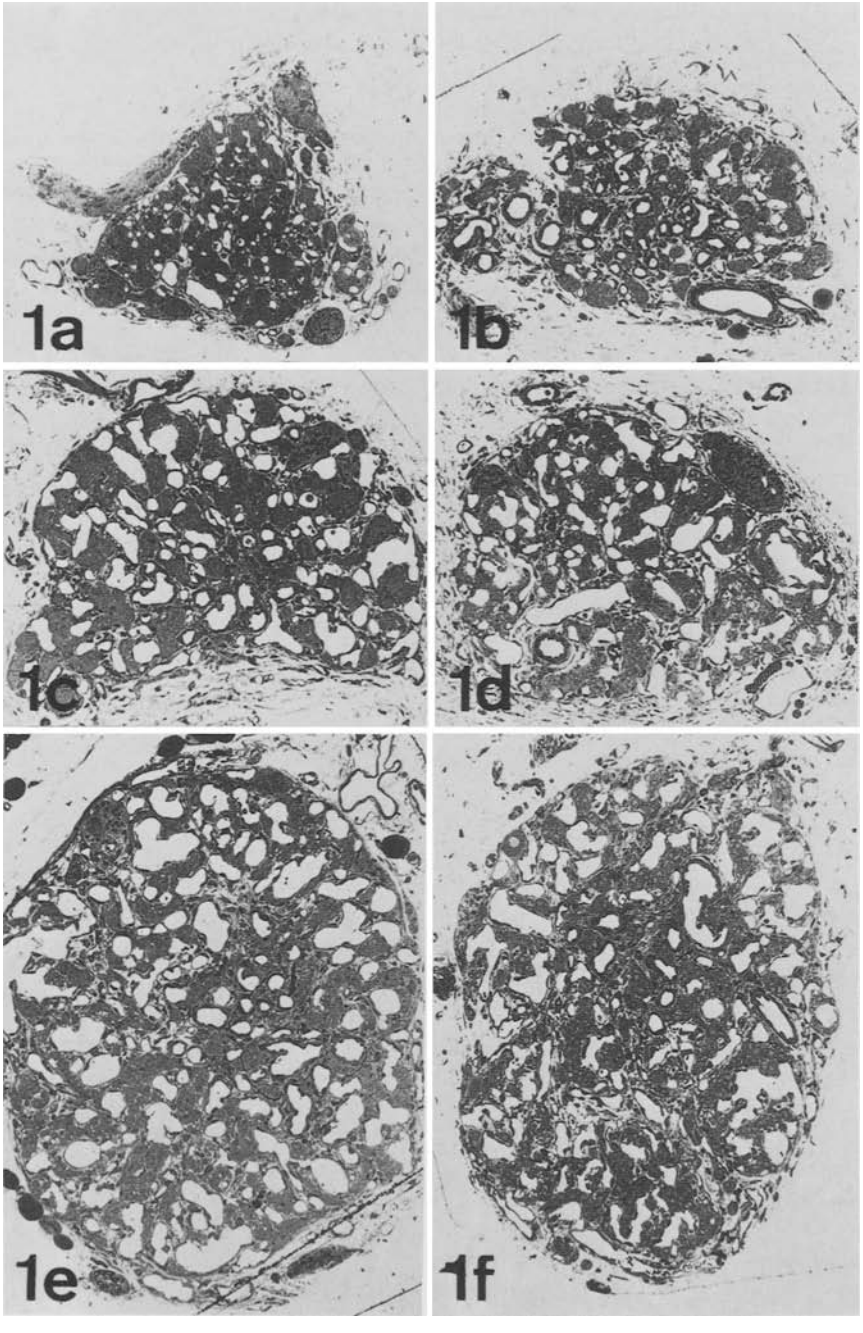

Fig. 1 a–h. Light microscopical pictures of intact (I) and sympathectomized (S) rat carotid bodies during control and hypoxic conditions. Toluidine-blue stained semithin sections. × 100. **a** I, control; **b** S, control; **c** I, hypoxia 1 week; **d** S, hypoxia 1 week; **e** I, hypoxia 2 weeks; **f** S, hypoxia 2 weeks; **g** I, hypoxia 3 weeks; **h** S, hypoxia 3 weeks

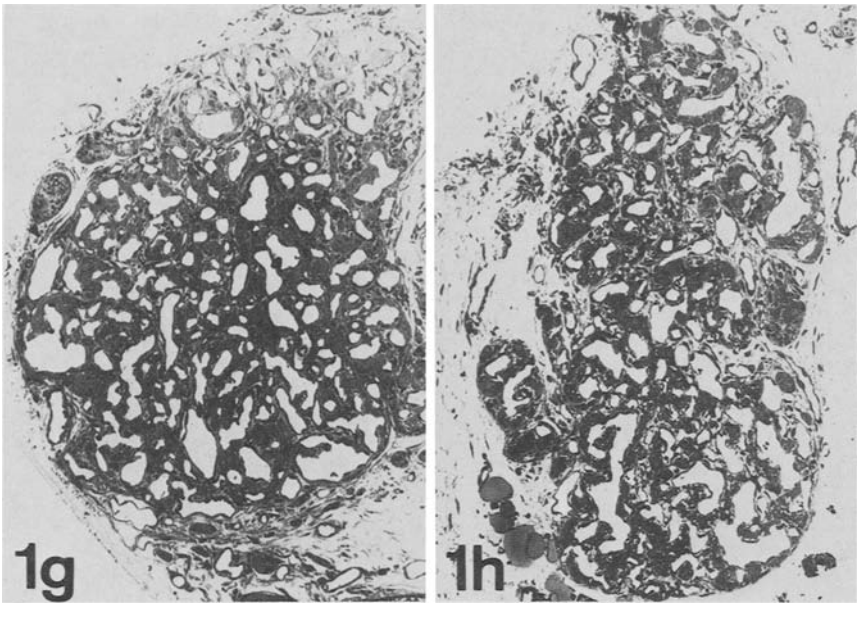

Table 1. Effects of long-term hypoxia on the structure of rat carotid body. Light microscopical morphometric data showing volume densities of the different cell constituents. The *left* carotid bodies are *sympathectomized* in all animals *except in intact controls*. The *right* carotid bodies are *intact* in all animals

Experimental group	Volume densities (%)							
	Type I, Type II cells		Blood vessel lumen		Endothelial cells		Interstitial tissue	
	left	right	left	right	left	right	left	right
Controls								
Intact	45.1±2.1	48.2±1.9	17 ±0.25	15.1±1.8	5.4±0.5	6.1±0.6	32.4±1.9	30.5±1.8
Sympathectomized	68.3±3	—	14.3±1.7	—	4.6±0.2	—	42.4±1.5[a]	—
Hypoxia								
1 week	32.6±1.3[b]	37.3±1.2	24.1±2.6[a]	27.3±1.75[a]	6.5±0.7[a]	5.1±0.6	36.7±2.1[a,b]	30.3±1.8
2 weeks	37.9±1.8	34.9±1.8[a]	28.7±0.9[a,b]	35.1±0.7[a]	5.3±0.4	5 ±0.6	28.1±2.8[a]	24.9±1.8[a]
3 weeks	30.1±2.9	33.1±1.9[a]	28.2±3.2[a]	31.6±2.6[a]	6.4±0.7[a]	6.5±0.4	35.3±4.2[a]	28.7±0.7

Values in per cent expressed as mean ±SE ($n=5$, except for sympathectomized controls where $n=4$).

[a] $P<0.05$ sympathectomized controls vs. intact controls, hypoxia vs. normoxia

[b] $P<0.05$ sympathectomized carotid bodies vs. intact carotid bodies

total volume of carotid bodies is enhanced from the first week of exposure to hypoxia and reaches its maximal level within 2 weeks. Then there is about a 4-fold increase in carotid body size.

The major change in tissue constituents is the constant increase in blood vessel lumen. The capillary volume is enhanced 4- and 9-fold after 1 and

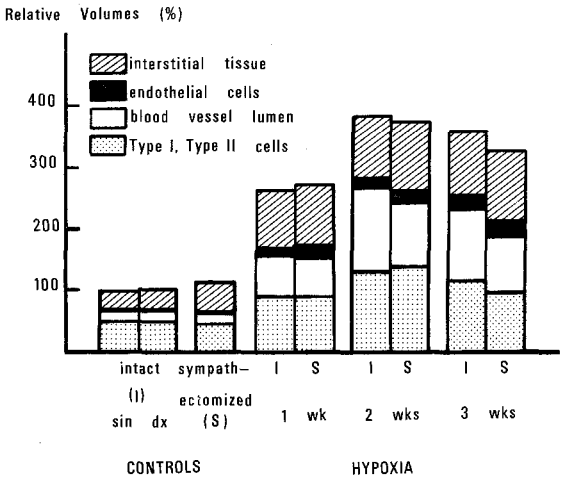

Fig. 2. Mean volumes of tissue constituents in carotid bodies of control and hypoxic rats. The volumes are expressed as percentages of the intact control level

2 weeks of hypoxia respectively. But long-term hypoxia induces modifications in volumes of other constituents too. Thus there is a 2- and 3-fold increase in the mean volume occupied by endothelial cells within 1 and 2 weeks respectively. The total volumes of glomic cells as well as interstitial tissue are enhanced too, about 2 times after 1 week and 3 times after 2 weeks of hypoxia. Any change in total volumes between 2 and 3 weeks cannot be detected. Ganglionectomy does not modify the quantitative structural changes observed in hypoxia compared to those in intact carotid bodies (Fig. 2).

Discussion

The present morphometric study confirms that the major component of the enlargement of carotid bodies is a greatly increased vascularity as previously reported by Blessing and Wolff (1973) and Laidler and Kay (1975a). From 1 week of hypoxia, this change seems to be caused both by vasodilatation and an ingrowth of new blood vessels as indicated by the increase in endothelial cell volume. Subsequently a new architecture of the vasculature develops as at least a part of the newly formed capillaries appear inside the glomic islands, a feature not observed in controls. These findings might reflect functional changes of the organ where an increasing capillary network is of primary importance for adjusting the carotid body to an increased metabolic activity during long-term hypoxia. Remodelling of the vascular architecture might be one method by which this chemoreceptor organ alters its sensitivity. While the vascular changes seem to plateau after 2 weeks of exposure to hypoxia, biochemical alterations still occur as the content of catecholamines in carotid body is still increased between 2 and 4 weeks of hypoxia (Hanbauer et al. 1981). These biochemical events might indicate largely delayed inductions of enzymes responding to hypoxia (Hanbauer et al. 1977). The mechanism whereby blood vessels are dilated is

not known. Dopamine which is released from carotid body during hypoxia (Hanbauer and Hellström 1978) is a vasodilative agent (Goldberg 1972). Though the existence of dopamine receptors on the carotid body blood vessels has not been studied the question arises whether dopamine may elicit the vasodilatation caused by chronic hypoxia.

In addition to the vascular changes, the volume occupied by glomic cells (type I, type II cells) is increased during long-term hypoxia. Whether this is due to hypertrophy and/or hyperplasia of the glomic cells and if one of the two types of glomic cells is selectively affected cannot be determined from the present data. In a study on 10 hypoxic rats, Laidler and Kay (1975) observed hyperplasia of type I cells in 4 rats only. Using stereological methods at an ultrastructural level, these authors demonstrated an increase in cytoplasmic volume of type I cells (Laidler and Kay 1978) thus indicating hypertrophy. In attempts to get a conclusive answer to these important questions an ultrastructural evaluation of the present experimental material is in progress.

It is not possible to judge whether the changes of volume of glomic cells can account for the tremendously increased storing capacity of catecholamines known to occur from 1 to 4 weeks of hypoxia (Hanbauer et al. 1981). At 2 weeks the increase in catecholamines is 10-fold while the volume of glomic tissue is enhanced 4 times only. This discrepancy between the biochemical and the light microscopical findings may be explained by the hypoxic-induced changes in ultrastructure of type I cells as suggested by some preliminary data (Hellström and Pequignot 1982). According to the size of amine-containing dense-cored vesicles, two kinds of type I cells, large vesicle cells and small vesicle cells, can be distinguished in normoxic carotid bodies of rat (Hellström 1975; Mc Donald and Mitchell 1975). During long-term hypoxia the catecholamine storage capacity of glomus cells is increased by two means, first and mainly by a conversion of small vesicle cells to large vesicle cells and then by an increase in total number of dense-cored vesicles (Hellström and Pequignot 1982).

Another feature of long-term hypoxic carotid bodies is the increase in volume of interstitial tissue in a proportion similar to that of glomic cells as previously noted by Laidler and Kay (1975a). The interstitial stroma in the carotid body contains many collagenous fibers but practically no elastic fibers (Verna 1979). The development of fibrous connective tissue may be associated with the further requirements in support and nutrition of hypertrophic glomic tissue.

Removal of the superior cervical ganglion does not influence the structural changes induced by long-term hypoxia compared to intact carotid bodies. Most of the sympathetic fibres innervating the carotid body, seem to terminate on blood vessels. These fibres can mediate vasoconstriction and consequently an increase in the chemosensory discharge (Belmonte and Eyzaguirre 1974). The present experiments provide evidence that the metabolic alterations induced by sympathectomy in long-term hypoxic rats (Hanbauer et al. 1981) cannot be attributed to a modification in local blood-flow as sympathectomy fails to enlarge the blood vessel lumen in control and hypoxic carotid bodies.

These results do not exclude an influence of sympathetic nerve activity on the morphology of vascular walls. In small pulmonary arteries of mouse long-term hypoxia leads to a hyperplasia of the smooth muscle fibres of the tunica media (Naeye 1965). Alpha-methyl-dopa, a sympatholytic drug, abolishes this hypoxic-induced vascular change. However it should be noted that, in carotid bodies, only arteries and arterioles are surrounded by smooth muscle cells. There are pericytes and no muscle cells around the capillaries (Verna 1979).

The possibility of direct control of glomus cell activity by noradrenergic nerves exists. Sympathetic fibres innervating type I cells have been shown (see for review McDonald 1981; Verna 1979) and might regulate the chemoafferent activity (O'Reagan 1977). Although the features of glomic cells at a light microscopical level seem to be unaffected by sympathectomy, ultrastructural studies may reveal some changes of e.g. the size and distribution of the vesicles in type I cells during long-term hypoxia. Such studies are in progress.

Acknowledgements. This work was supported by a fellowship to J.M.P. from the Svenska Institutet and by grants from the Swedish Medical Research Council (B81-12X-05663-02).

References

Arias-Stella J (1969) Human carotid body at high altitudes. Am J Pathol 55:82a–83b

Barer GR, Edwards C, Jolly AI (1972) Changes in the ventilatory response to hypoxia and in carotid body size in chronically hypoxic rats. J Physiol (Lond) 221:27P–28P

Belmonte C, Eyzaguirre C (1974) Efferent influences on carotid body chemoreceptors. J Neurophysiol 37:1131–1143

Blessing MH, Wolff H (1973) Befunde and Glomus caroticum der Ratte nach Aufenthalt in einer simulierten Höhe von 7500 m. Virchows Arch [Pathol Anat] 360:79–92

Dunnill MS (1968) Quantitative methods in histology. In: Dyke SC (ed) Recent advances in clinical pathology. Churchill, London, p 401

Edwards CW (1971) The carotid body in animals at high altitude. In: Porter R, Knight J (eds) High altitude physiology: Cardiac and respiratory aspects. Ciba Foundation Symposium. Churchill, London, pp 79–88

Goldberg LI (1972) Cardiovascular and renal actions of dopamine: potential clinical applications. Pharmacol Rev 24:1–29

Hanbauer I, Hellström S (1978) The regulation of dopamine and noradrenaline in the rat carotid body and its modification by denervation and by hypoxia. J Physiol (Lond) 282:21–34

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 6:81–86

Hanbauer I, Lovenberg W, Costa E (1977) Induction of tyrosine-3-mono-oxygenase in carotid body of rats exposed to hypoxic conditions Neuropharmacology 16:277–282

Hellström S (1975) Morphometric studies of dense-cored vesicles in Type I cells of rat carotid body. J Neurocytol 4:77–86

Hellström S, Koslow SH (1975) Biogenic amines in carotid body of adult and infant rats – a gas chromatographic – mass spectrometric assay. Acta Physiol Scand 93:540–547

Hellström S, Pequignot JM (1982) Morphometric studies on intact and sympathectomized carotid bodies of long-term hypoxic rats. A light and electron microscopical study. In: Pallot D (ed) Proc VII Int Conf on Arterial Chemoreceptors. Helm Ltd, Leicester

Laidler P, Kay JM (1975a) A quantitative morphological study of the carotid bodies of rats living at a simulated altitude of 4300 metres. J Pathol 117:183–191

Laidler P, Kay JM (1975b) The effect of chronic hypoxia on the number and nuclear diameter of type I cells in the carotid bodies of rats. Am J Pathol 79:311–318

Laidler P, Kay JM (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. J Neurocytol 7:183–192

Lewis LD, Pontén U, Siesjö BK (1973) Arterial acid-base changes in unaesthetized rats in acute hypoxia. Respir Physiol 19:312–321

McDonald DM (1981) Peripheral chemoreceptors. Structure – function relationships of the carotid body. In: Hornbein TF (ed) Regulation of breathing, pt 1. Dekker Inc, New York, pp 105–319

McDonald DM, Mitchell RA (1975) The innervation of glomus cells, ganglion cells and blood vessels in the rat carotid body: A quantitative ultrastructural analysis. J Neurocytol 4: 177–230

Naeye RL (1965) Effect of alpha-methyl-dopa on heart and pulmonary arteries of hypoxic mice. Am J Physiol 209:702–704

O'Reagan RG (1977) Control of carotid body chemoreceptors by autonomic nerves. Irish J Med Sci 146:199–205

Purves MJ (1970) The role of the cervical sympathetic nerve in the regulation of oxygen consumption of the carotid body of the cat. J Physiol (Lond) 209:417–431

Verna A (1979) Ultrastructure of the carotid body in the mammals. Int Rev Cytol 60:271–330

Weibel ER (1969) Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol 26:235–302

Accepted March 18, 1983