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Peptidergic innervation in the rat carotid body after 2, 4, and 8 weeks of hypocapnic hypoxic exposure

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Summary. The distribution and abundance of neuropeptide-containing nerve fibers were examined in the carotid bodies of rats exposed to hypocapnic hypoxia (10% O_2 in N_2) for 2, 4, and 8 weeks. The carotid bodies after 2, 4, and 8 weeks of hypoxic exposure were enlarged by 1.2-1.5 times in the short axis, and 1.3-1.7 times in the long axis in comparison with the normoxic control ones. The enlarged carotid bodies contained a number of expanded blood vessels. Mean density per unit area (10^4 μm^2) of substance P (SP) and calcitonin gene-related peptide (CGRP) immunoreactive fibers was transiently high in the carotid bodies after 4 weeks of hypoxic exposure, and decreased significantly to nearly or under 50% after 8 weeks of hypoxic exposure. Density of vasoactive intestinal polypeptide (VIP) immunoreactive fibers increased significantly in all periods of hypoxic exposure observed, and was especially high in the carotid bodies after 4 weeks of hypoxic exposure. Density of neuropeptide Y immunoreactive fibers was unchanged in the carotid bodies during hypoxic exposure. These characteristic changes in the density of SP, CGRP, and VIP fibers in the carotid bodies after 4 weeks of hypoxic exposure suggest that the role of these neuropeptide-containing fibers may be different in the carotid bodies after each of three periods of hypoxic exposure, and that the peptidergic innervation after 8 weeks of hypoxic exposure may show an acclimatizing state.

Key words: Hypoxic exposure, Carotid body, Regulatory neuropeptides, Immunohistochemistry, Rat

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Introduction

It has been reported by many authors that the carotid bodies, which are the primary organs for sensing changes in arterial blood gases (PaO_2 and $PaCO_2$) and hydrogen ion concentration, are enlarged in rats exposed to long term hypoxia. The volume of the carotid bodies increases several fold with increased vascularization (Heath et al., 1973; Laidler and Kay, 1975a,b; Barer et al., 1976; Kusakabe et al., 1993, 1998a,b, 2000, 2002), and the glomus cells (type I cells and chief cells) show hypertrophy and hyperplasia (Moller et al., 1974; Laidler and Kay, 1978; Pequignot and Hellström, 1983; Dhillon et al., 1984; McGregor et al., 1984; Pequignot et al., 1984; Pallot et al., 1990). As a result of enlargement with vascular expansion, the appearance of the rat hypoxic carotid bodies becomes similar to those of the amphibians (Kusakabe et al., 1993), whose PaO_2 is generally low (Toews and Heisler, 1982; West et al., 1987).

In the enlarged carotid bodies of rats exposed for 3 months to systemic (hypocapnic-, isocapnic-, and hypercapnic-) hypoxia, we recently demonstrated the changes in the peptidergic innervation by means of immunohistochemistry (Kusakabe et al., 1998, 2000, 2002). In the chronically hypocapnic- and isocapnic hypoxic carotid bodies, the density per unit area of parenchymal vasoactive intestinal polypeptide (VIP)-immunoreactive fibers increased significantly, the density of substance P (SP) and calcitonin gene-related peptide (CGRP) immunoreactive fibers decreased significantly to under 50%, and the density of neuropeptide Y (NPY)-immunoreactive fibers was unchanged in comparison with the normoxic controls (Kusakabe et al., 1998, 2000). On the other hand, in the chronically hypercapnic hypoxic carotid body, the density of NPY fibers was significantly increased, and that of VIP was unchanged (Kusakabe et al., 2002). This indicates that the density of VIP and NPY fibers in the

chronically hypercapnic hypoxic carotid bodies was opposite to that in hypocapnic and isocapnic hypoxia. Thus, our recent findings in systemic hypoxia have suggested that long term (3 months) hypoxic exposure changes the abundance of peptidergic fibers in the rat carotid body, and that the different levels of arterial CO_2 tension also change the peptidergic innervation during chronic hypoxic exposure. However, it is possible that altered peptidergic innervation in the long term (3 months) hypoxic carotid bodies shows a completely acclimatized state. To clarify this, it is necessary to perform an identical survey in this organ after different periods of hypoxic exposure.

In the present study, we examined the changes in the distribution and abundance of four different regulatory neuropeptides, SP, CGRP, VIP, and NPY in the carotid bodies of rats exposed to hypocapnic hypoxia for 2, 4, and 8 weeks. We also examined the morphological changes in the carotid body, especially those in the vasculature, during each of the three periods of hypoxia to evaluate the different levels of hypoxic exposure.

Materials and methods

Chronically hypoxic exposure

Eight-week-old rats were placed in an air-tight acrylic chamber with two holes. One hole, located at the top of a side wall of the chamber, was connected to a multi-flowmeter (MODEL-1203, KOFLOC, Japan), and was used to deliver a hypoxic gas mixture (10% O_2 in N_2: total 20 L/min) into the chamber. The flow of air and N_2 was regulated by a multi-flowmeter, and the O_2 and the CO_2 levels within the box were monitored with a gas analyzer (Respina 1H26, NEC San-ei, Japan). The second hole was located at the bottom of the opposite wall of the chamber and was used to flush out the gas mixture. The temperature within the chamber was maintained at 25 °C. This hypoxic condition was confirmed to be hypocapnic to rats in our previous study (Hirakawa et al., 1997). Animals were exposed in this chamber for 2, 4, and 8 weeks with food and water available *ad libitum*. At least three rats of each of three hypoxic periods were examined. Control rats were housed for each of the three periods (2, 4, and 8 weeks) in the same chamber ventilated by air at the same flow rate. The chamber was opened for 10 min every 3 days for husbandry.

All experiments with animals were performed in accordance with the "Principles of laboratory animal care" (NIH publ. no. 86-23, revised 1985) and with the "Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences" published by the Physiological Society of Japan.

Tissue preparation

The animals were intraperitoneally anesthetized with sodium pentobarbital (0.05 mg/g), and perfused through a thin nylon tube inserted into the ventricle with 0.1M heparinized phosphate buffer saline (PBS), followed by freshly prepared Zamboni's fixative solution (4% paraformaldehyde and 0.2% picric acid in 0.1M PBS) at a constant flow rate. The pair of carotid bodies was then removed under a dissecting microscope, and immersed in the same fixative for an additional 6-8 h at 4 °C. After a brief washing in PBS, the specimens were transferred to 30% sucrose in PBS at 4 °C for 24 h. The specimens were cut serially at 16 μm on a cryostat, and mounted in four series on poly-L-lysine-coated slides.

Immunohistochemistry

The sections were processed for immunohistochemistry according to the peroxidase-antiperoxidase (PAP) method. The immunostaining procedure has been detailed in a previous report (Kusakabe et al., 1991). In brief, the sections were incubated at 4 °C overnight with the primary polyclonal antisera against the following neuropeptides: SP (1:1500; Cambridge Research Biochemicals, Northwich, UK); CGRP (1:1500; Cambridge Research Biochemicals, Northwich, UK); VIP (1:2000; Incstar, Stillwater, USA), and NPY (1:2000; Incstar, Stillwater, USA). The peroxidase activity was demonstrated with 3,3'-diaminobenzidine. The reaction for neuropeptides was verified by treating sections with primary antibody which had been inactivated by overnight incubation with 50-100 μM of its peptide. Some sections were also stained with hematoxylin and eosin for general histology.

Data analysis

In hematoxylin and eosin-stained sections through the center of the carotid bodies, their short and long axis and the diameter of blood vessels were measured with an ARGUS 100 computer and image processor (Hamamatsu-Photonics, Japan). The measurement was performed on 6 sections taken from 6 carotid bodies of 3 rats exposed to hypoxia and exposed to air for each of the three periods. The values taken from hypoxic carotid bodies were expressed as means ± SD (n=6), and those from normoxic control ones were also expressed as means ± SD (n=18) because there was no significant difference between the values obtained from the normoxic control carotid bodies of the rats housed for each of the three periods (2, 4, and 8 weeks) in the chamber ventilated with air. The number of blood vessels of seven different ranges of diameter, less than 5 μm (~5), 6-10 μm (~10), 11-15 μm, (~15) 16-20 μm (~20), 21-25 μm (~25), 26-30 μm (~30), and 31-35 μm (~35), in normoxic control and hypoxic carotid bodies was expressed as percentage of total number of blood vessels.

The density of immunoreactive fibers in the normoxic and hypoxic carotid bodies was represented as the number of varicosities per unit area (10^4 μm^2) of

parenchyma. The manner of measurement is detailed in our recent reports (Kusakabe et al., 1998b, 2000, 2002). The number of varicosities was counted on 6 sections of hypoxic carotid bodies after each of three hypoxic exposures, and on 18 sections from normoxic control carotid bodies.

The values were expressed as means ± SD, and statistical comparisons between the control and experimental values were determined using Student's t-test.

Results

General histology of the normoxic carotid bodies and those after 2, 4, and 8 weeks of hypocapnic hypoxic exposure

In hematoxylin and eosin-stained sections through the center of the normoxic rat carotid bodies, the bodies were oval in shape and were mainly composed of clusters of glomus cells and vessels with narrow lumens (Fig. 1A). The mean short and long axes of the normoxic carotid bodies were 329.0±3 5.8 μm and 439.7±28.5 μm, respectively. These values are expressed as the means of 18 carotid bodies obtained from 9 rats, because there was no significant difference between the values obtained from the normoxic control carotid bodies of the rats housed for each of three periods (2, 4, and 8 weeks) in the chamber ventilated with air.

The carotid bodies of the hypocapnic hypoxic rats were found to be enlarged several fold in comparison with those of normoxic control rats (Fig. 1B-D), as recently reported (Kusakabe et al., 2000). However, the rate of enlargement was different for the carotid bodies exposed for 2, 4, and 8 weeks. In the sections through the center of the enlarged hypoxic carotid bodies, the mean short and long axes of the carotid bodies of the rats exposed to hypoxia were 377.1±30.0 μm and 555.1±27.2 μm for 2 weeks, were 453.1±37.7 μm and 728.4±44.6 μm for 4 weeks, and were 484.6±91.3 μm and 760.9±29.9 μm for 8 weeks, respectively. The mean short axis of the carotid bodies of the rats exposed to hypoxia for 2, 4, and 8 weeks was 1.2 (377.1/329.0 μm), 1.3 (453.1/329.0 μm), and 1.5 (484.6/329.0 μm) times larger than in normoxic controls, respectively (Fig. 2). The mean long axis was 1.3 (555.1/439.7 μm), 1.6 (728.4/439.7 μm), and 1.7 (760.9/439.7 μm) times larger than in normoxic controls, respectively (Fig. 2). All values in the hypoxic carotid bodies were significantly ($p<0.01$ or $p<0.005$) larger than those in the normoxic control ones.

In the normoxic control carotid bodies, about 48.9% and 44.3% of the blood vessels were small ones with diameters less than 5 μm and 10 μm, respectively. The percentage of relatively large vessels with diameters greater than 16 μm was less than 5% (Fig. 3A). In the carotid bodies after 2 weeks of hypoxic exposure, the percentage of small blood vessels with diameters less than 5 μm and 10 μm decreased from 48.9 to 28.7% and from 44.3 to 37.4%, respectively, and that of relatively large vessels with diameters greater than 16 μm increased to over 10% (Fig. 3B). In the carotid bodies after 4 and 8 weeks of hypoxic exposure, the percentage of small vessels with diameters less than 5 μm further decreased to under 20%, that of diameters greater than 16 μm further increased to over 20%, and a few large vessels with diameters greater than 30 μm were observed (Fig. 3C,D). Thus, the ratio of vascular expansion in the carotid bodies after 4 and 8 weeks of hypoxic exposure was greater than in the carotid bodies after 2 weeks of exposure.

Peptidergic nerve fibers in the normoxic carotid bodies

Immunoreactivity of four different neuropeptides, SP, CGRP, VIP, and NPY, was recognized in the nerve fibers distributed throughout the parenchyma of the carotid body (Figs. 4A, 5A, 6A, 7A) as recently reported (Kusakabe et al., 1998b, 2000, 2002). These immunoreactive fibers appeared as thin processes with a number of varicosities. NPY-immunoreactive varicose fibers were more numerous than SP-, CGRP-, and VIP-immunoreactive fibers. Most of them were associated with the vessels within the carotid body. The mean density of varicosities of SP, CGRP, VIP, and NPY fibers per unit area (10^4 μm^2) was 6.1±0.8, 14.8±1.8, 13.1±2.2, and 45.7±4.8, respectively. No glomus cells with the immunoreactivity of these four neuropeptides were observed in the normoxic controls.

Peptidergic nerve fibers in the carotid bodies after 2, 4, and 8 weeks of hypocapnic hypoxic exposure

The distribution pattern of SP-, CGRP-, VIP-, and NPY- immunoreactive fibers in the carotid bodies after 2, 4, and 8 weeks of hypocapnic hypoxic exposure was similar to that in the normoxic ones. Most immunoreactive fibers were associated with the blood vessels. There were, however, some differences in the abundance of SP, CGRP, and VIP fibers in the carotid bodies after each of the three periods of hypoxic exposure (Figs. 4A-D, 5A-D, 6A-D, 7A-C). When the mean density of varicosities per unit area (10^4 μm^2) was compared between the normoxic controls and the carotid bodies after 2 weeks of hypoxic exposure, the density of VIP fibers was significantly ($p<0.005$) increased from 13.1±2.2 to 20.7±3.6, although there was no significant difference in the density of SP, CGRP, and NPY fibers (Fig. 8). In the same way, when the mean density of four neuropeptide-containing fibers was compared between the control carotid bodies and those after 4 weeks of exposure, the density of SP ($p<0.005$), CGRP ($p<0.01$), and VIP fibers ($p<0.005$) was significantly increased from 6.1±0.8 to 9.7±2.0, 14.8±1.8 to 19.2±4.7, and 13.1±2.2 to 33.1±6.6, respectively (Fig. 8). That of NPY fibers was unchanged (Fig. 8). Furthermore, when the density of these two peptidergic fibers was compared between the control carotid bodies and those after 8

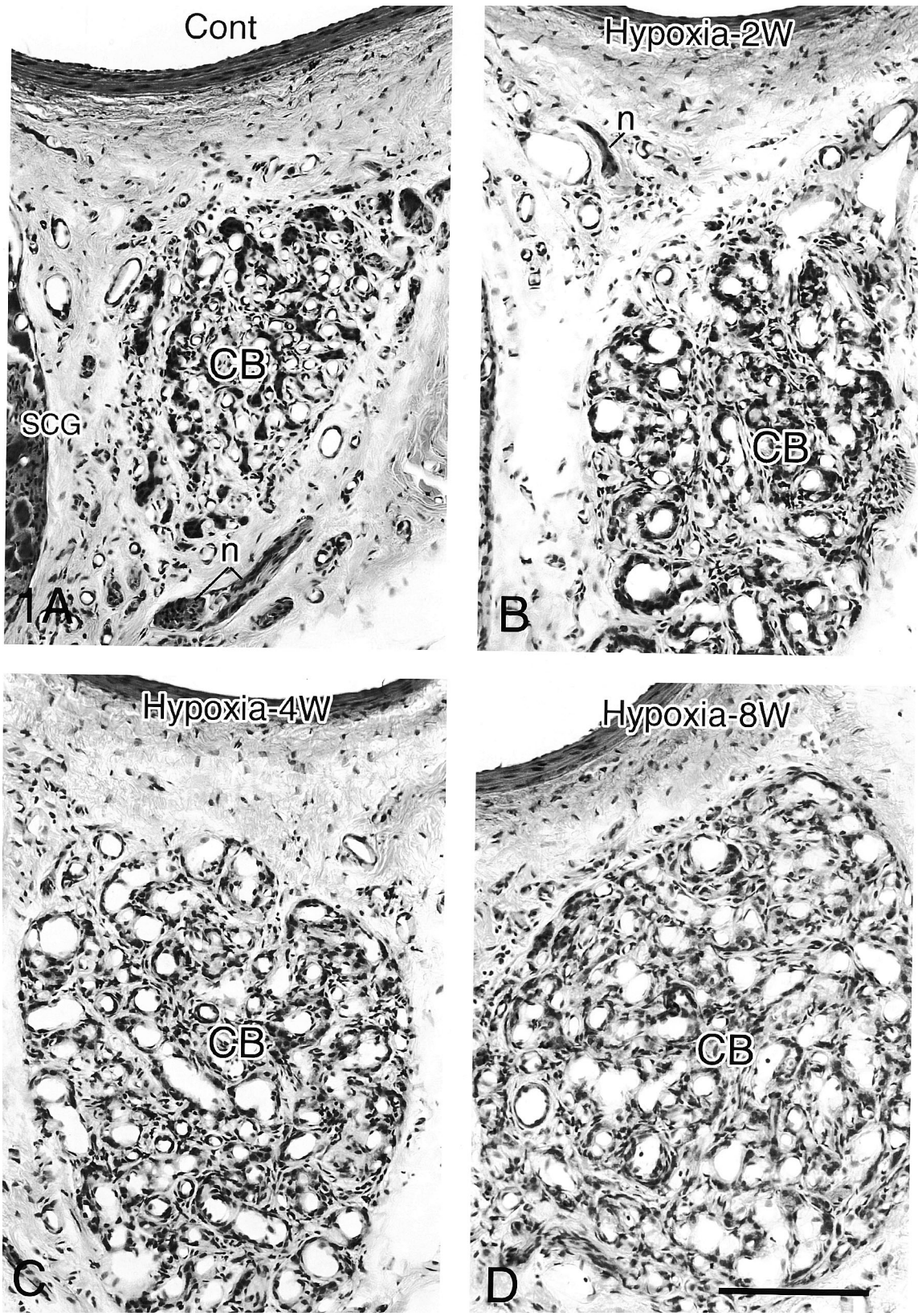

Fig. 1. Hematoxylin-eosin stained sections from the center of a control normoxic carotid body (CB) **(A)**, and a carotid body after 2 **(B)**, 4 **(C)**, and 8 weeks **(D)** of hypocapnic hypoxic exposure. The hypoxic carotid bodies (CB) are enlarged with vascular expansion in comparison with normoxic control (Cont). SCG: superior cervical ganglion. Scale bar: 200 μm.

weeks of exposure, the density of SP and CGRP fibers was significantly ($p<0.005$) decreased from 6.1±0.8 to 3.1±0.5 and from 14.8±1.8 to 9.4±0.9, respectively (Fig. 8), and when the mean density of these peptidergic fibers was compared between the carotid bodies after 4 weeks and 8 weeks of exposure, the density of SP and CGRP fibers was significantly ($p<0.005$) decreased from 9.7±2.0 to 3.1±0.5 and from 19.2±4.7 to 9.4±0.9, respectively (Fig. 8). VIP fibers in the carotid bodies after 8 weeks of hypoxic exposure decreased in comparison with those after 4 weeks of exposure, but the density of VIP fibers after 8 weeks of exposure was higher than in the control carotid bodies. The density of NPY fibers was unchanged during hypoxic exposure (Fig. 8). There were no glomus cells with the immunoreactivity of these four neuropeptides in the carotid bodies after 2, 4, or 8 weeks of hypocapnic hypoxic exposure.

Discussion

The present study demonstrates that the carotid bodies of the rats exposed to hypocapnic hypoxia for each of three different periods, 2, 4, and 8 weeks, were significantly enlarged, 1.2-1.5 times in the short axis, and 1.3-1.7 times in the long axis, in comparison with the normoxic control ones. With a prolonged hypoxic exposure, the percentage of blood vessels with relatively narrow lumens, less than 5-10 μm, decreased, and that with relatively wide lumens, more than 21 μm, increased. Thus, the enlargement of the hypoxic carotid bodies was mainly due to vascular dilation as previously suggested (Blessing and Wolff, 1973; Laidler and Kay, 1975a,b; Pequignot and Hellström, 1983). Pequignot and Hellström (1983) reported that vascular dilation is already evident in the carotid bodies after 1 week of exposure to hypoxia, and that the volume density of blood vessel lumens seems to reach a plateau after 2 weeks of exposure to hypoxia. In their work, 4-week-old rats were used, but we used 8-week-old rats. This differing age of animals at the start of hypoxia and the experimental conditions of hypoxic exposure may be the cause of the difference described above. In either case, it seems likely that the enlargement of the carotid body with vascular expansion begins soon after the start of hypoxic exposure.

As stated above in the introduction, most authors reported several-fold enlargement of the carotid bodies in rats exposed to long term hypoxia. In these studies,

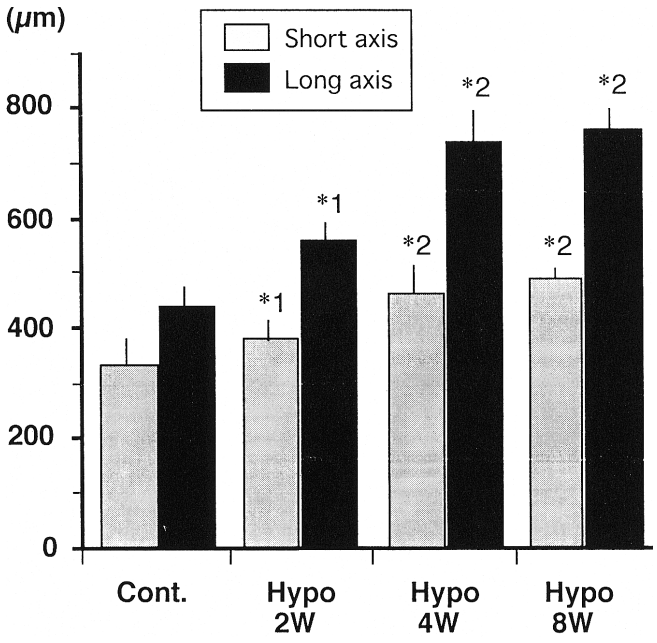

Fig. 2. Histograms comparing the short and long axes of normoxic control carotid bodies and those after 2, 4, and 8 weeks of hypoxic exposure. *1: $p<0.01$, and *2: $p<0.005$ in comparison with the control column.

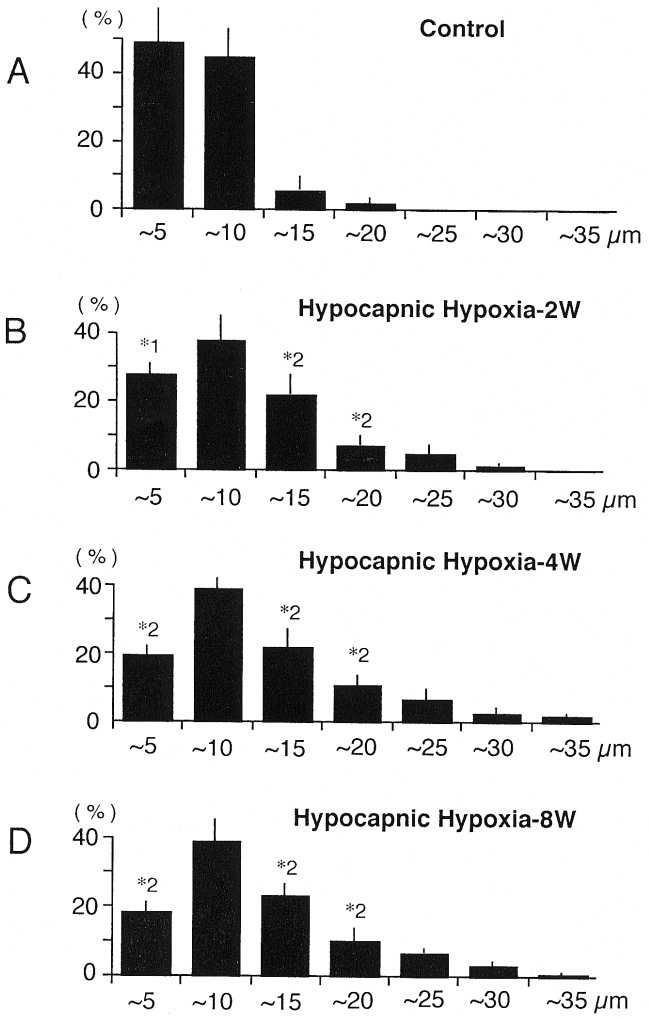

Fig. 3. Histograms representing the percentage of blood vessels of seven ranges of diameter in normoxic control carotid bodies **(A)** and those after 2 **(B)**, 4 **(C)**, and 8 weeks **(D)** of hypoxic exposure.

the duration of hypoxic exposure varied. In some studies the animals were exposed to hypoxia for relatively short periods of 1-2 weeks, and in other studies for relatively long periods of 2-3 months. However, most authors use the term "chronic hypoxia" in their publications. This terminology can cause much confusion. The present study on the carotid bodies after 2, 4, and 8 weeks showed that all carotid bodies after hypoxic exposure are enlarged with vascular expansion although the amount of enlargement is different for each. Thus, as far as enlargement of the carotid bodies is concerned, the use of the term "chronic hypoxia" might have little meaning as a general expression regardless of the duration of hypoxic exposure.

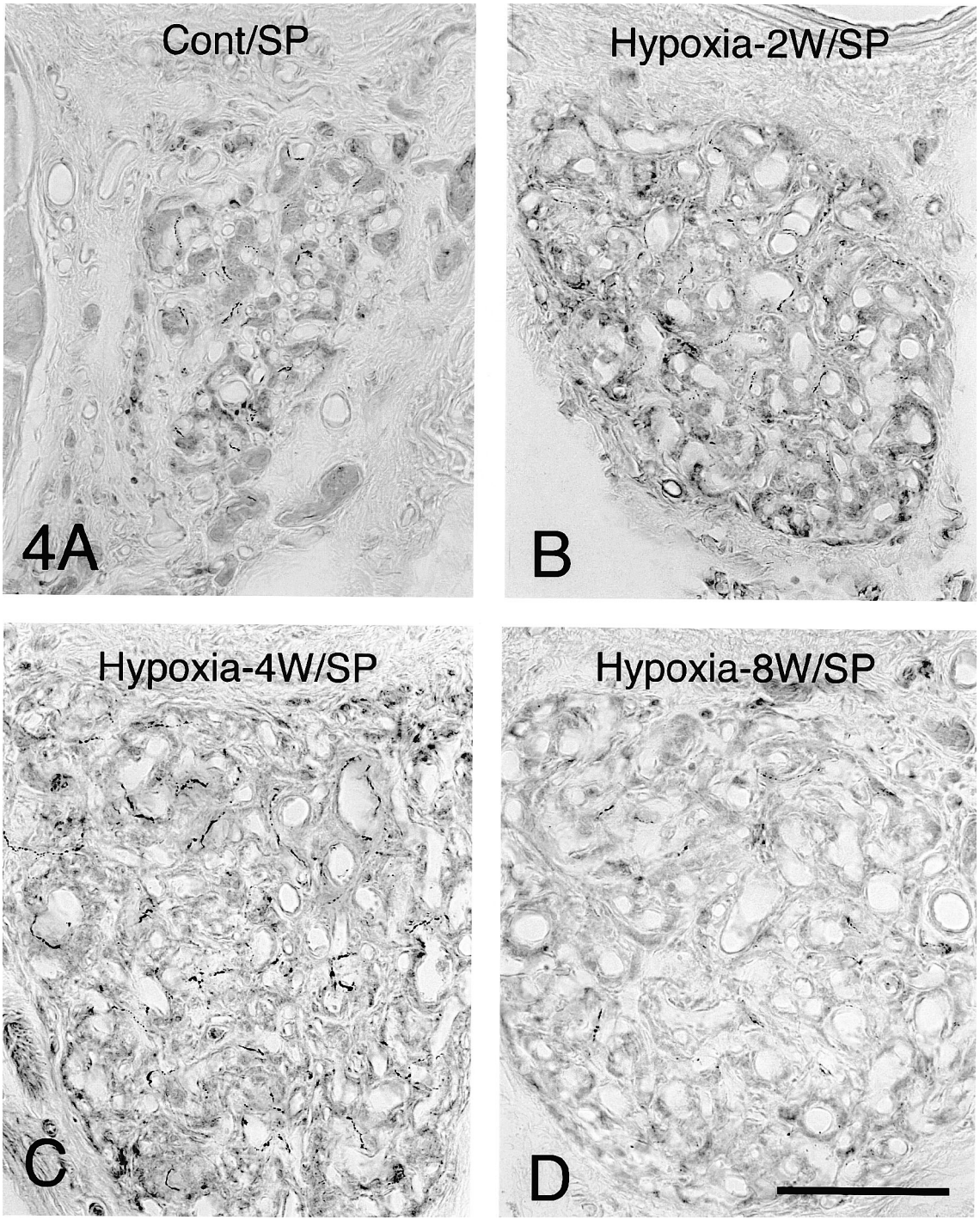

Fig. 4. SP immunoreactive nerve fibers in a control carotid body (Cont) **(A)**, and those after 2 **(B)**, 4 **(C)**, and 8 weeks **(D)** of hypoxic exposure. The density of SP fibers is higher in the carotid body after 4 weeks of exposure than in the control (Cont) and the other two periods of hypoxic exposure. Scale bar: 200 μm.

On the other hand, in the case of changes in the peptidergic innervation, the different duration of hypoxic exposure becomes an important subject of discussion. During hypoxic exposure, the most striking feature of the peptidergic innervation in the carotid bodies is their transiently increased density of SP and CGRP fibers after 4 weeks of hypoxic exposure. Thereafter, the density of SP and CGRP fibers in the carotid bodies after 8 weeks of hypoxic exposure significantly decreased to nearly or under 50%, as recently reported in the carotid bodies after 3 months of hypoxic exposure (Kusakabe et al., 2000). This transient change in SP and CGRP fibers makes us suppose a further reliable hypothesis for the role of SP and CGRP fibers in the hypoxic carotid

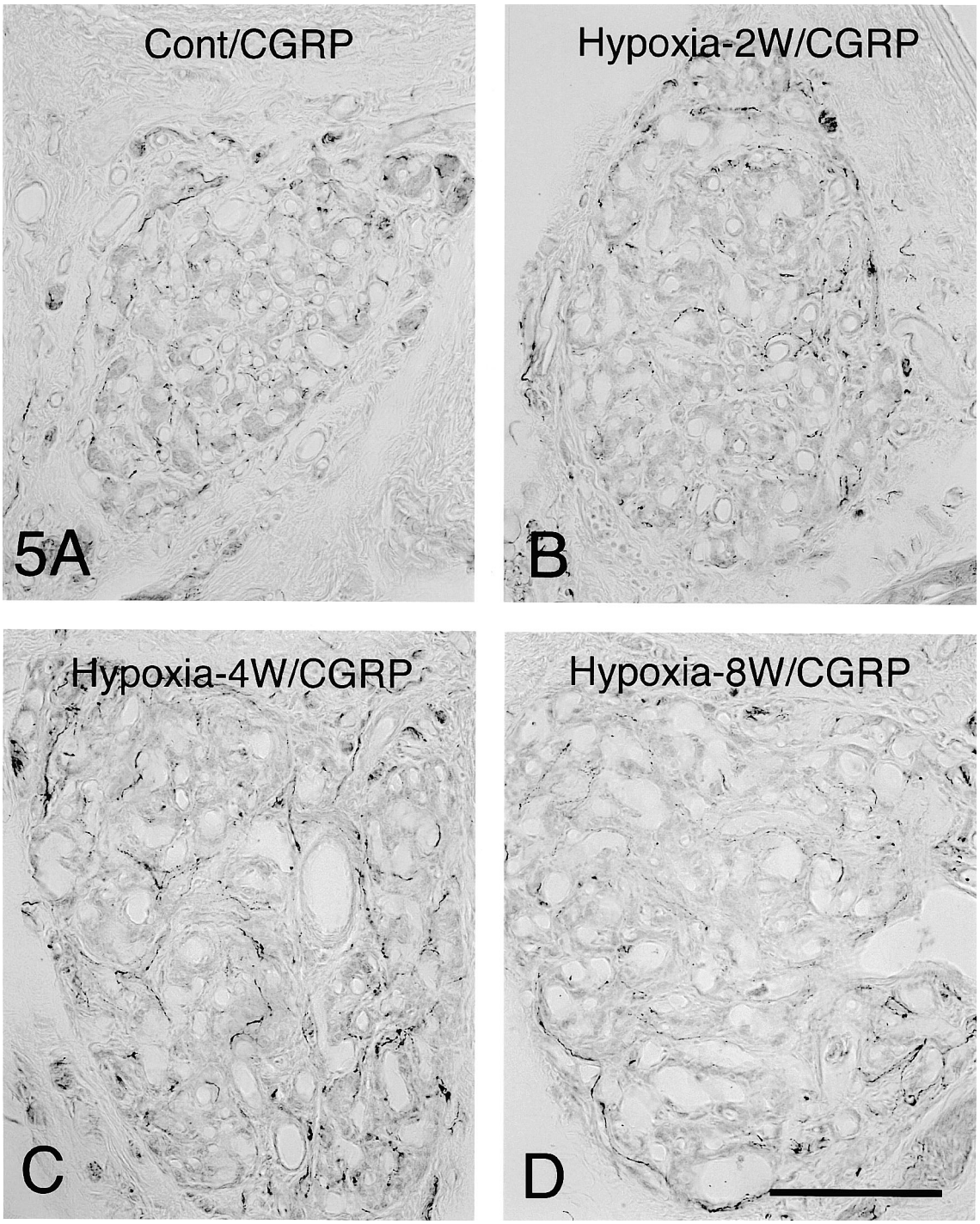

Fig. 5. CGRP immunoreactive nerve fibers in a control carotid body (Cont) **(A)**, and those after 2 **(B)**, 4 **(C)**, and 8 weeks **(D)** of hypoxic exposure. Many CGRP fibers are found in the carotid body after 4 weeks of exposure. Scale bar: 200 μm.

bodies, although we recently proposed that SP and CGRP fibers in carotid bodies after 3 months of hypoxic exposure may not be involved in chemosensory mechanisms because the density of these fibers at this stage decreases to under 50% (Kusakabe et al., 2000). It has been reported that SP and CGRP containing fibers in the mammalian carotid body are involved in chemoreceptor mechanisms (Helke et al., 1980; Jacobowitz and Helke, 1980; Wharton et al., 1980; Lundberg and Hökfelt, 1983) because SP and CGRP fibers in the carotid body originate from the sensory jugular and petrosal ganglia (Chen et al., 1986).

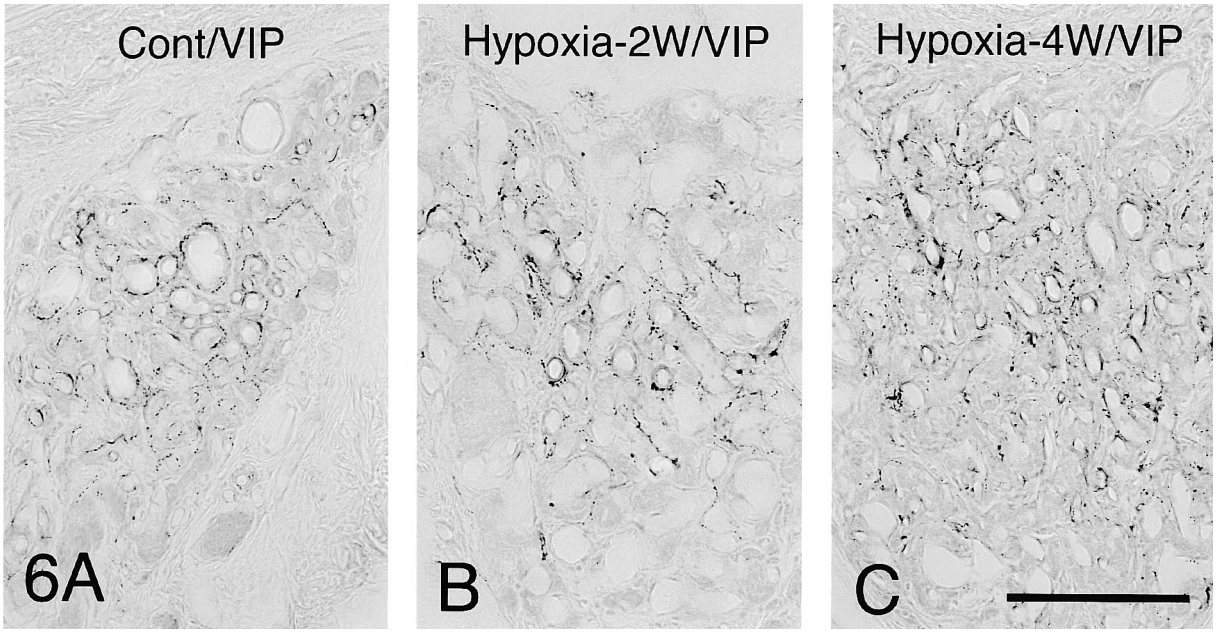

Fig. 6. VIP immunoreactive nerve fibers in a control carotid body (Cont) **(A)**, and those after 2 **(B)** and 4 weeks **(C)** of hypoxic exposure. Most VIP fibers are associated with blood vessels. Scale bar: 200 μm.

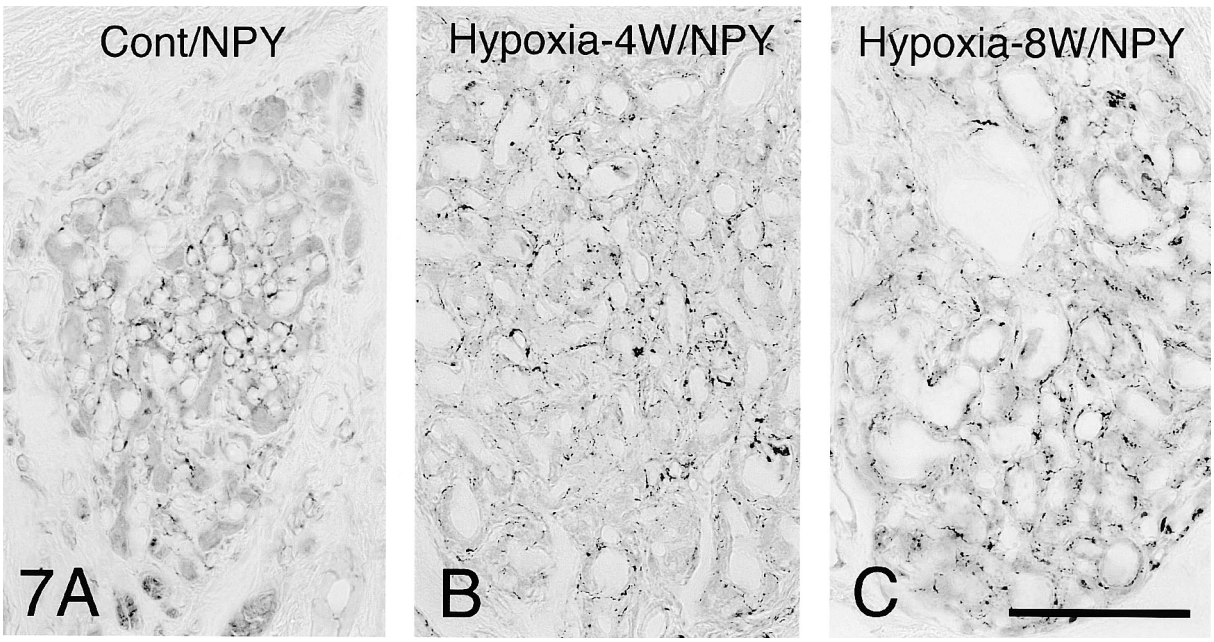

Fig. 7. NPY immunoreactive nerve fibers in the control carotid body (Cont) **(A)**, and those after 4 **(B)** and 8 weeks **(C)** of hypoxic exposure. The abundance of NPY fibers is unchanged during hypoxic exposure. Scale bar: 200 μm.

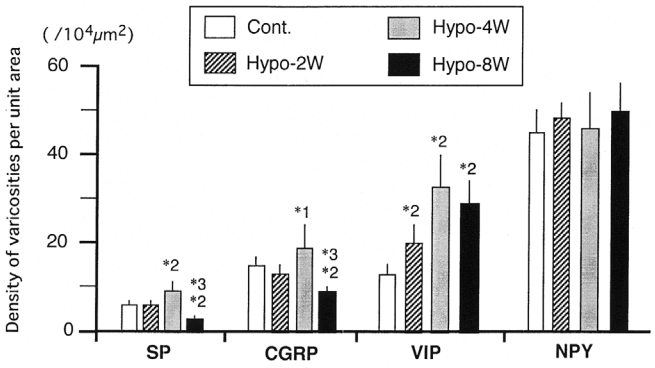

Fig. 8. Histogram comparing the density of varicosities per unit area in normoxic control carotid bodies (Cont), and those after 2, 4, and 8 weeks hypoxic exposure. *1: p<0.01, and *2: p<0.005 in comparison with the control column, and *3: p<0.005 in comparison with the 4-week column.

Furthermore, McQueen (1980) reported a dose-dependent increase in chemosensory discharge after SP injection to the carotid body, and Cragg et al. (1994) suggested that SP is associated with chemosensory stimulation. On this basis, SP and CGRP fibers in the carotid bodies after 4 weeks of hypoxic exposure may be involved in the chemosensory system. On the other hand, there is a possibility that the efferent component of the glossopharyngeal nerve possesses SP and CGRP because SP and CGRP are thought to have a vasodilatory effect (Hallberg and Pernow, 1975; Samnegard et al., 1978; Edvinsson et al., 1981; Edvinsson and Uddman, 1982; Brain et al., 1985) in addition to a sensory role. Accordingly, SP and CGRP fibers in the carotid bodies after 4 weeks of hypoxic exposure are also involved in vascular dilation in this hypoxic period of the carotid body. We suggest again that SP and CGRP fibers in the carotid bodies after 4 weeks of hypoxic exposure may be involved in both chemosensory and vascular dilatory systems.

The density of VIP fibers in the carotid bodies after each of three periods of hypoxic exposure is higher than in controls, and is especially higher than in carotid bodies after 4 weeks of exposure. As far as we are aware, there are no reports suggesting that VIP is concerned in the chemosensory mechanisms, and VIP is also thought to have a long acting vasodilatory effect (Wilson et al., 1981). Therefore, in a recent report, we strongly suggested that at least part of the vascular expansion in 3-month hypoxic carotid bodies may depend on the vasodilatory effect of VIP, and concluded that VIP fibers are indirectly involved in chemosensory mechanisms by controlling local carotid body circulation. We also suggest that the vasodilation of VIP is more effective in the carotid bodies after 4 weeks of exposure than in those after longer exposure.

The physiological role of NPY in chemosensory mechanisms is not completely understood. Previous studies have shown an increased chemosensory activity caused by stimulation of the sympathetic nerve to the carotid body, and the effect is generally considered to result from vasoconstriction in the carotid body (Eyzaguirre et al., 1983). In various mammalian vasculatures, NPY is thought to have a vasoconstrictory effect (Lundberg et al., 1982; Edvinsson et al., 1983; Brain et al., 1985). In addition to VIP, SP, and CGRP fibers, NPY fibers may also be involved in the regulation of local carotid body circulation, although the effect of NPY is in the reverse direction. In the present study after 2, 4, and 8 weeks of hypoxic exposure, the density of NPY fibers was unchanged, as recently reported in the carotid bodies after 3 months exposure (Kusakabe et al., 2000). Consequently, it seems likely that physiological involvement of NPY fibers is invariable from short to prolonged hypoxic exposure.

Considered together with our recent results (Kusakabe et al., 2000), the peptidergic innervation after 8 weeks of hypoxic exposure may show an acclimatizing state, and the peptidergic innervation after 3 months of exposure indicates a completely acclimatized state. As stated above, the use of the term "chronic hypoxia" may have little meaning as far as enlargement of the carotid bodies is concerned. In the case of changes in the peptidergic innervation, however, it is necessary to be careful about the use of the term "chronic hypoxia". From these points, the present results may provide a kind of standard for further studies of the hypoxic carotid bodies.

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References

Barer G.R., Edwards C. and Jolly A.I. (1976). Changes in the carotid body and the ventilatory response to hypoxia in chronically hypoxic rats. Clin. Sci. 50, 311-313.

Blessing M.H. and Wolff H. (1973). Befunde an glomus caroticum der ratte nach aufenhalt im einer simulierten höhe von 7500m. Virchow Arch. Pathol. Anat. Physiol. 360, 78-97.

Brain S.D., Williams T.J., Tippins J.R., Moris H.R. and MacIntyre I. (1985). Calcitonin gene-related peptide is a potent vasodilator, Nature (Lond) 313, 54-56.

Chen L., Yates R.D. and Hansen J.T. (1986). Substance P-like immunoreactivity in rat and cat carotid bodies: light and electron microscopic studies. Histol. Histopathol. 1, 203-212.

Cragg P.A., Runold M., Kou Y.R. and Prabhakar N.R. (1994). Tachykinin antagonists in carotid body responses to hypoxia and substance P in the rat. Respir. Physiol. 95, 295-310.

Dhillon D.P., Barer G.R. and Walsh M. (1984). The enlarged carotid

body of the chronically hypoxic and chronically hypoxic and hypercapnic rat: A morphometric analysis. Quart. J. Exp. Physiol. 69, 301-317.

Edvinsson L. and Uddman R. (1982). Immunohistochemical localization and dilatatory effect of substance P on human cerebral vessels, Brain Res. 232, 466-471.

Edvinsson L., McCulloch J. and Uddman R. (1981). Substance P: immunohistochemical localization and effect upon feline pial arteries in vitro and in situ, J. Physiol. (Lond) 318, 251-258.

Edvinsson L., Emson P., McCulloch J., Teramoto K., and Uddman R. (1983). Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat, Neurosci. Lett. 43, 79-84.

Eyzaguirre C., Monti-Bloch L., Hayashida Y. and Baron M. (1983) Biophysics of the carotid body receptor complex. In: Physiology of the peripheral arterial chemoreceptors . Acker H. and O'Regan R.G. (eds). Elsevier. Amsterdam. pp 59-87.

Hallberg D. and Pernow B. (1975). Effect of substance P on various vascular beds in the dog. Acta Physiol. Scand. 93, 277-285.

Heath D., Edwarda C., Winson M. and Smith P. (1973). Effects on the right ventricle, pulmonary vasculature, and carotid bodies of the rat of exposure to, and recovery from, simulated high altitude. Thorax 28, 24-28.

Helke C.J., O'Donohue T.J. and Jacobowitz D.M. (1980). Substance P as a baro- and chemoreceptor afferent neurotransmitter: immunohistochemical and neurochemical evidence in the rat. Peptides 1, 1-9.

Hirakawa H., Nakamura T. and Hayashida Y. (1997). Effect of carbon dioxide on autonomic cardiovascular responses to systemic hypoxia in conscious rats. Am. J. Physiol. 273, R747-R754.

Jacobowitz D.M. and Helke C.J. (1980). Localization of substance P immunoreactive nerves in the carotid body. Brain Res. Bull. 5, 195-197.

Kusakabe T., Anglade P. and Tsuji S. (1991). Localization of substance P, CGRP, VIP, neuropeptide Y, and somatostatin immunoreactive nerve fibers in the carotid labyrinths of some amphibian species. Histochemistry 96, 255-260.

Kusakabe T., Powell F.L. and Ellisman M.H. (1993). Ultrastructure of the glomus cells in the carotid body of chronically hypoxic rats: with special reference to the similarity of amphibian glomus cells. Anat. Rec. 237, 220-227.

Kusakabe T., Matsuda H., Harada H., Hayashida Y., Gono Y., Kawakami T. and Takenaka T. (1998a). Changes in the distribution of nitric oxide synthase immunoreactive nerve fibers in the chronically hypoxic rat carotid body. Brain Res. 795, 292-296.

Kusakabe T., Hayashida Y., Matsuda H., Gono Y., Powell F.L., Ellisman M.H., Kawakami T. and Takenaka T. (1998b). Hypoxic adaptation of the peptidergic innervation in the rat carotid body. Brain Res. 806, 165-174.

Kusakabe T., Hayashida Y., Matsuda H., Kawakami T. and Takenaka T. (2000). Changes in the peptidergic innervation of the carotid body a month after the termination of chronic hypoxia. Adv. Exp. Med. Biol. 475, 793-799.

Kusakabe T., Hirakawa H., Matsuda H., Yamamoto Y., Nagai T., Kawakami T., Takenaka T. and Hayashida Y. (2002). Changes in the peptidergic innervation in the carotid body of rats exposed to hypercapnic hypoxia: an effect of arterial CO_2 tension. Histol. Histopathol. 17, 21-29.

Laider P. and Kay J.M . (1975a). A quantitative morphological study of the carotid bodies of rats living at a simulated altitude of 4300 meters. J. Pathol. 117, 183-191.

Laider P. and Kay J.M. (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, 311-320.

Laider P. and Kay J.M. (1978). A quantitative study of some ultrastructural features of the type I cells in carotid bodies of rats living at a simulated altitude of 4300 meters. J. Neurocytol. 7, 183-192.

Lundberg J.M. and Hökfelt T. (1983). Coexistence of peptides and classical neurotransmitters. Trends Neurosci. 6, 325-333.

Lundberg J.M., Terenis L., Hökfelt T., Martling C.R., Tatemoto K., Mutt V., Polak J., Bloom S. and Goldstei M. (1982). Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol. Scand. 116, 477-480.

McGregor K.H., Gil J. and Lahiri S. (1984). A morphometric study of the carotid body in chronically hypoxic rats. J. Appl. Physiol. 57, 1430-1438.

McQueen D.S. (1980). Effects of substance P on carotid chemoreceptor activity in the cat. J. Physiol. (Lond) 302, 31-47.

Moller M., Mollgard K. and Sorensen S.C. (1974). The ultrastructure of the carotid body in chronically hypoxic rabbits. J. Physiol. (Lond). 238, 447-453.

Pallot D.J., Bee D., Barer G.R. and Jacob S. (1990). Some effects of chronic stimulation on the rat carotid body, In: Arterial chemoreception. Eyzaguirre C., Fidone S.J., Fitzgerald R.S., Lahiri S. and McDonald D.M. (eds). Springer-Verlag. New York. pp 293-301.

Pequignot J.M. and Hellström S. (1983). Intact and sympathectomized study of the carotid bodies of long term hypoxic rats: A morphometric light microscopical study. Virchow Arch. Pathol Anat. 400, 235-243.

Pequignot J.M., Hellström S. and Johansson C. (1984). Intact and sympathectomized study of the carotid bodies of long term hypoxic rats: A morphometric ultrastructural study. J. Neurocytol. 13, 481-493.

Samnegard H., Thulin L., Tyden G., Johansson C., Muhrberg O. and Björklund C. (1978). Effect of synthetic substance P on internal carotid artery blood flow in man. Acta Physiol. Scand. 104, 492-495.

Toews S.P. and Heisler N. (1982). The effects of hypercapnia on intracellular and extracellular acid-base status in the toad *Bufo marinus*. J. Exp. Biol. 97, 79-86.

West N.H., Topor Z.L. and Van Vliet B.N. (1987). Hypoxemic threshold for lung ventilation in the toad. Respir. Physiol. 70, 377-390.

Wharton J., Polak J.M., Pearse A.G.E., McGregor G.P., Bryant M.G., Bloom S.R., Emson P.C., Bisgard G.E. and Will J.A. (1980). Enkephalin-, VIP- and substance P-like immunoreactivity in the carotid body. Nature 284, 269-271.

Wilson D.A., O'Neil J.T., Saido S.I. and Traystman R.J. (1981). Vasoactive intestinal polypeptide and canine cerebral circulation. Circ. Res. 48, 138-148.

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