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Review

Hypoxic adaptation of the rat carotid body

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Summary. Three types of hypoxia with different levels of carbon dioxide (hypocapnic, isocapnic, and hypercapnic hypoxia) have been called systemic hypoxia. The systemic hypoxic carotid bodies were enlarged several fold, but the degree of enlargement was different for each. The mean short and long axes of hypocapnic and isocapnic hypoxic carotid bodies were 1.6 (short axis) and 1.8-1.9 (long axis) times larger than normoxic control carotid bodies, respectively. Those of hypercapnic hypoxic carotid bodies were 1.2 (short axis) and 1.5 (long axis) times larger than controls, respectively. The rate of enlargement in hypercapnic hypoxic carotid bodies was lower than in hypocapnic and isocapnic hypoxic carotid bodies. The rate of vascular enlargement in hypercapnic hypoxic carotid bodies was also smaller than in hypocapnic and isocapnic hypoxic carotid bodies. Thus, the enlargement of hypoxic carotid bodies is mainly due to vascular dilation. Different levels of arterial CO_2 tension change the peptidergic innervation during chronically hypoxic exposure. The characteristic vascular arrangement was under the control of altered peptidergic innervation. During the course of hypoxic adaptation, the enlargement of the carotid bodies with vascular expansion began soon after the start of hypoxic exposure. During the course of recovery, the shrinking of the carotid bodies with vascular contraction also started at a relatively early period after the termination of chronic hypoxia. These processes during the course of hypoxic adaptation and during the course of recovery were under the control of peptidergic innervation. These findings may provide a standard for further studies of hypoxic carotid bodies.

Key words: Carotid body, Hypoxia, Adaptation, Recovery, Immunohistochemistry, Regulatory neuropeptides, Rat

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Introduction

It is generally accepted 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, become 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., 1993b, 1998a,b, 2000, 2002, 2003, 2004b), 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 that of amphibians (Kusakabe et al., 1993b; Kusakabe, 2002), whose PaO_2 is generally low (Toews and Heisler, 1982; West et al., 1987).

In early studies, experimental animals were exposed to hypoxia equivalent to that of a high altitudes (3800-7500 m) in a hypobaric chamber (Blessing and Wolff, 1973; Heath et al., 1973; Moller et al., 1974; Laidler and Kay, 1975a,b, 1978; Barer et al., 1976), and in later studies, the animals were also exposed to hypoxia in a normobaric hypoxic chamber (10% O_2) (Pequignot and Hellström, 1983; Dhillon et al., 1984; McGregor et al., 1984; Pequignot et al., 1984; Kusakabe et al., 1998a,b; 2000, 2002, 2003, 2004b).

Looking back on a number of previous hypoxic studies, we must emphasize that the condition and duration of experimental hypoxic exposure varied with each author, so that it is difficult to compare them. In brief, most studies on hypoxic exposure have not referred to carbon dioxide levels except in a few instances (Dhillon et al., 1984). Furthermore, some authors exposed the animals to hypoxia for short periods of 1-2 weeks, and other authors for long periods of 2-3 months. However, most authors use the term "chronic hypoxia" in their publications. When a number of previous findings are compared, the various hypoxic conditions and durations in each experiment will be in

danger of being misunderstood. In recent years, we have tried to examine the morphological changes in the rat carotid bodies in various hypoxic conditions and durations to provide a morphological standard (Kusakabe et al., 1998b, 2000, 2002, 2003, 2004b).

In this review, we first summarize the morphological changes in the rat carotid bodies in three types of hypoxia (hypocapnic, isocapnic, and hypercapnic hypoxia) with different levels of carbon dioxide to evaluate different levels of arterial CO_2 tension, and then summarize the morphological changes in the rat carotid bodies during the course of hypoxic adaptation, and during the course of recovery to evaluate the different levels of acclimatization and deacclimatization. We also summarize the changes in the peptidergic innervation in systemic hypoxia and in the course of hypoxic adaptation and recovery.

Table 1. Mean values of PaO_2, $PaCO_2$, and pH in the systemic hypoxic rats (n=6). (Hayashida et al., 1996).

	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH
Control (n=6)	94.2	34.3	7.43
Hypocapnic hypoxia(n=6)	38.7	21.5	7.56
Isocapnic hypoxia (n=6)	50.5	35.4	7.43
Hypercapnic hypoxia (n=6)	56.4	45.0	7.33

General morphology in rat carotid body in systemic hypoxia

The modulating effect of CO_2 on the circulatory response to chronic hypoxia in sinoaortic denervated dogs assesses the arterial chemoreflex contribution (Koehler et al., 1980). Elevating $PaCO_2$ attenuated tachycardia during hypoxia. This may indicate the interaction between the modulating effects of increased CO_2 level on cardiovascular responses and the carotid body chemoreceptor reflex. It is meaningful to discuss the effects of hypoxia which could be induced by varying levels of carbon dioxide, because recently there has been much concern over global warming caused by

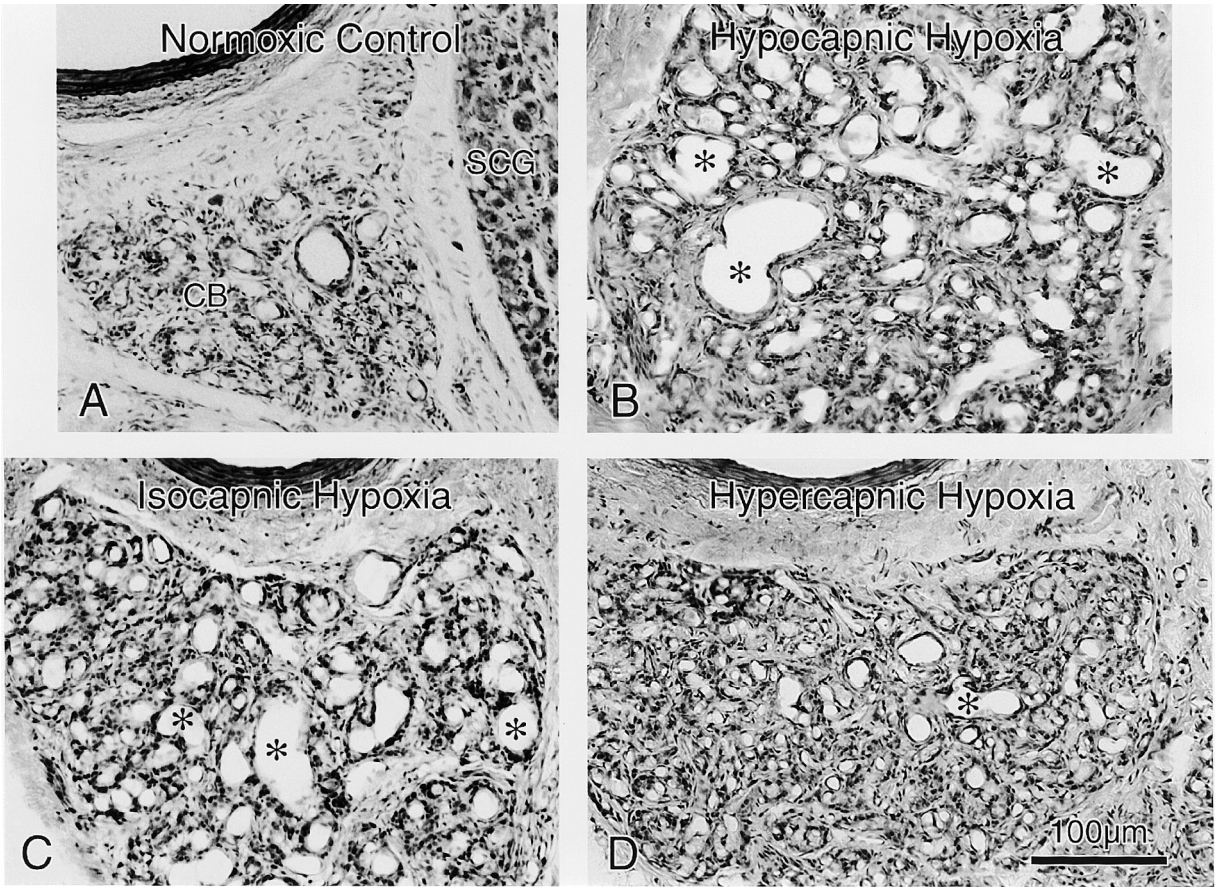

Fig. 1. A comparison of hematoxylin-eosin stained sections of a normoxic control carotid body (CB) **(A)**, and hypocapnic **(B)**, isocapnic **(C)**, and hypercapnic hypoxic carotid bodies **(D).** Asterisks (*) indicate expanded blood vessels. SCG: superior cervical ganglion. (Kusakabe et al., 1998b, 2002, 2003).

increased levels of this gas.

Three types of hypoxia with different levels of carbon dioxide (hypocapnic, isocapnic, and hypercapnic hypoxia) have been called systemic hypoxia, and the mean values of PaO_2, $PaCO_2$, and pH of the control and the three hypoxic groups are shown in Table 1 (Hayashida et al., 1996). Recently the changes in general morphology and those in peptidergic innervation in the carotid bodies of rats exposed to chronic (3 months) hypocapnic (10% O_2 in N_2), isocapnic (10% O_2 and 2-3% CO_2), and hypercapnic hypoxia (10% O_2 and 6-7% CO_2) were examined to evaluate the effect of arterial CO_2 tension (Kusakabe et al., 1998b, 2000, 2002, 2004b).

In the control normoxic rats, the carotid bodies are oval and highly vascular (Fig. 1A) as widely demonstrated by a number of investigators. According to our morphometry, the mean short and long axes of the normoxic control carotid bodies are 329.0±35.3 µm and 439.7±28.5 µm, respectively (Fig. 2). The carotid bodies of systemic hypoxic rats were found to be enlarged several fold, but the degree of enlargement was different for each (Fig. 1B-D). The mean short and long axes of hypocapnic hypoxic carotid bodies were 518.7±37.1 µm and 782.7±92.2 µm, those of isocapnic hypoxic carotid bodies were 525.8±24.4 µm and 827.7±29.5 µm, and those of hypercapnic hypoxic carotid bodies were 390.6±37.9 µm and 664.5±59.6 µm, respectively (Fig. 2). The mean short and long axes of hypocapnic and isocapnic hypoxic carotid bodies were 1.6 (short axis) and 1.8-1.9 (long axis) times larger than control normoxic carotid bodies, respectively. Those of hypercapnic hypoxic carotid bodies were 1.2 (short axis) and 1.5 (long axis) times larger than controls, respectively. Thus, the rate of enlargement in hypercapnic hypoxic carotid bodies was significantly lower than the rate in hypocapnic and isocapnic hypoxic carotid bodies (Fig. 2).

In the normoxic control carotid bodies, about 90% of blood vessels were small with diameters of less than 10 µm, and blood vessels with diameters greater than 15 µm were under 10% (Fig. 3). In the hypocapnic and isocapnic hypoxic carotid bodies, the percentage of small blood vessels less than 5 µm was reduced to under 20%, and blood vessels with diameters greater than 15 µm increased to about 40% (Fig. 3). In the hypercapnic hypoxic carotid bodies, the percentage of relatively small vessels and that of relatively large vessels were similar to that in normoxic control carotid bodies (Fig. 3), although the carotid bodies themselves were significantly larger than in normoxic controls.

It has been generally suggested that CO_2 tension causes vasodilation in both central and peripheral vascular systems (Fensternacher and Rapoport, 1984). In hypercapnic hypoxic carotid bodies, however, the percentage of vascular enlargement is small. This may indicate that high CO_2 tension causes the carotid body vasculature to constrict. However, this finding may be restricted to the chronically hypoxic condition.

Recently we reported the contribution of the autonomic nervous system to the cardiovascular

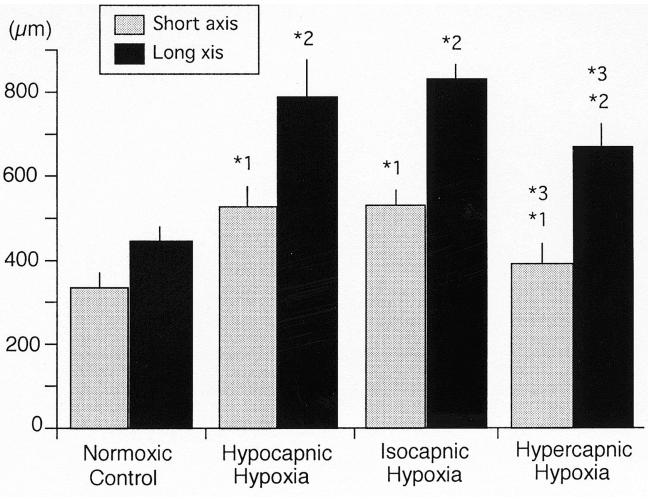

Fig. 2. Histograms comparing the diameter of normoxic control carotid bodies and systemic hypoxic carotid bodies. *1: p<0.005, and *2 p<0.005 in comparison with the normoxic control column. *3 p<0.005 in comparison with the hypocapnic and isocapnic hypoxic column. (Kusakabe et al., 2004a).

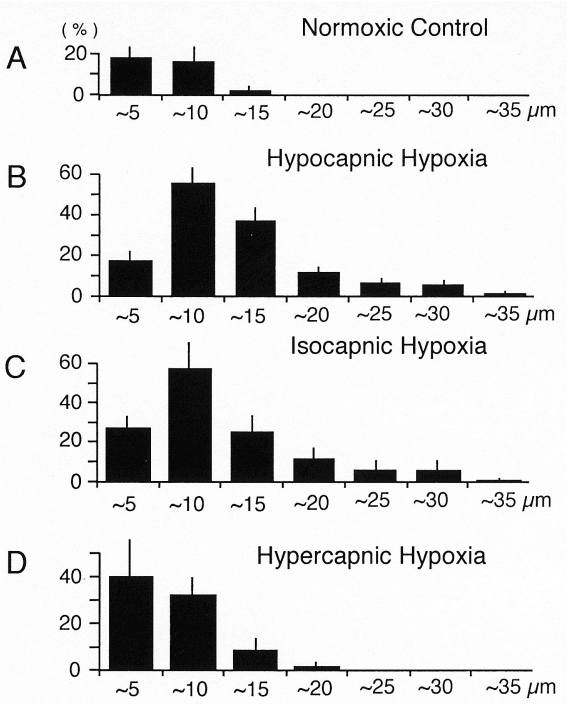

Fig. 3. Histograms representing the percentage of blood vessels of seven ranges of diameter in normoxic control carotid bodies **(A)** and hypocapnic **(B)**, isocapnic **(C)**, and hypercapnic hypoxic carotid bodies **(D)**. (Kusakabe et al., 2004a).

response to hypoxia with different levels of CO_2 in conscious rats (Hirakawa et al., 1997). Hypercapnic hypoxia causes an increase in arterial blood pressure, and a decrease in heart rate and renal sympathetic nerve activity, whereas hypocapnic hypoxia causes a decrease in arterial blood pressure, and an increase in heart rate and renal sympathetic nerve activity. Sino-aortic denervation and atropine abolished the bradycardic response during hypercapnic hypoxic exposure. On this basis, we suggested that hypercapnic hypoxia causes sympathetic and parasympathetic activation, while hypocapnic hypoxia causes sympathetic activation and parasympathetic inhibition. Furthermore, baroreflex function is affected by arterial CO_2 levels. From these findings, we suggested that cardiovascular responses during hypoxia are modified by arterial CO_2 levels, and mediated by the autonomic nervous system. Thus, different levels of arterial CO_2 tension may affect the autonomic nervous system.

Peptidergic innervation in rat carotid body in systemic hypoxia

In various vertebrates, many kinds of regulatory neuropeptides, substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), somatostatin, enkephalin, galanin, and others have been recognized in the nerve fibers distributed throughout the parenchyma of the carotid body (Lundberg et al., 1979; Wharton et al., 1980; Jacobowitz and Helke, 1980; Kondo et al., 1986; Kondo and Yamamoto, 1988; Kusakabe et al., 1991, 1993a,c, 1994a,b, 1995a,b, 1998b). Because these peptidergic fibers appeared as thin processes with a number of varicosities, we can easily represent the density of peptidergic innervation. In the normoxic control carotid bodies, the mean density of varicosities in SP, CGRP, VIP, and NPY immunoreactive fibers per unit area (10^4 μm^2) was 6.5±1.0, 14.3±1.6, 12.5±1.8, and 45.6±5.3, respectively (Fig. 4).

In the rat carotid bodies in systemic hypoxia, immunoreactivity of SP, CGRP, VIP, and NPY was also recognized in the same way as in the normoxic controls, although there were some differences in the abundance of immunoreactive fibers (Kusakabe et al., 1998b, 2000, 2002). NPY immunoreactive varicose fibers were more numerous than SP, CGRP, and VIP immunoreactive fibers. Most immunoreactive fibers were associated with the vasculature and some fibers surrounded clusters of glomus cells. A histogram comparing the mean density of varicosities in these neuropeptide-containing fibers per unit area (10^4 μm^2) is shown in Figure 4.

In the chronically isocapnic hypoxic carotid bodies, SP, CGRP, VIP, and NPY immunoreactive fibers were mainly associated with enlarged vasculature (Kusakabe et al., 1998b). In paticular, most VIP fibers were found around the vasculature. When the mean density of varicosities per unit area (10^4 μm^2) was compared in the same way, the density of VIP fibers was significantly increased from 12.5±1.8 to 22.6±1.6, although that of NPY fibers was unchanged (Fig. 4). The mean density of SP and CGRP fibers per unit area was significantly reduced from 6.5±1.0 to 2.2±0.3, and from 14.3±1.6 to 6.7±1.2, respectively (Fig. 4). The mean density of VIP fibers per unit area in chronically hypoxic carotid bodies was 1.8 (22.6/12.5) times higher than that of VIP fibers in normoxic carotid bodies, and the mean density of SP and CGRP fibers per unit area was 0.4 (2.2/6.5) and 0.5 (6.7/14.3) times higher than that of SP and CGRP fibers, respectively.

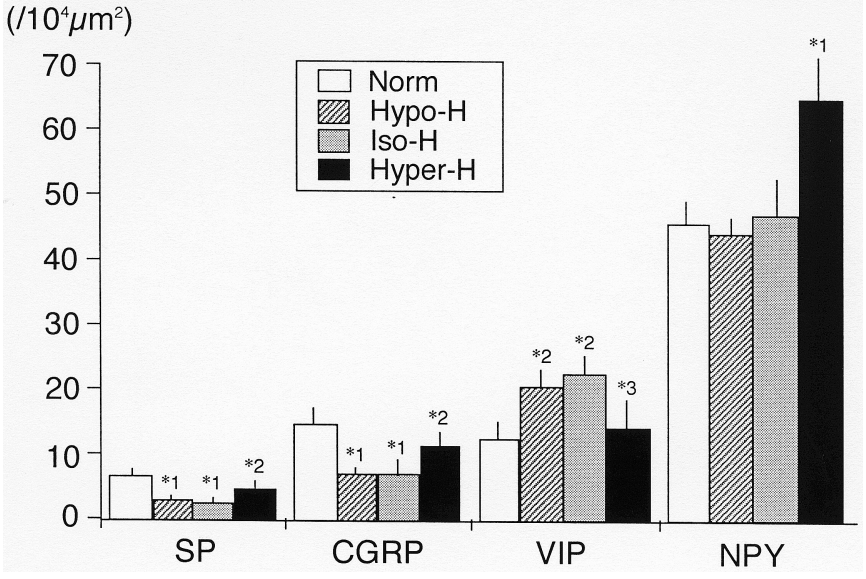

Fig. 4. Histograms comparing the density of varicosities of SP, CGRP, VIP, and NPY immunoreactive fibers per unit area in normoxic control and hypocapnic, isocapnic, and hypercapnic hypoxic carotid bodies. (Kusakabe et al., 2004a).

In chronically hypocapnic hypoxic carotid bodies, the density of VIP fibers was increased significantly 1.4 times, the density of SP and CGRP fibers was reduced significantly to under 50%, and the density of NPY was unchanged (Fig. 4) (Kusakabe et al., 2000). Thus, changes in peptidergic innervation in chronically hypocapnic hypoxic carotid bodies were similar to changes in chronically isocapnic hypoxic bodies.

In chronically hypercapnic hypoxic carotid bodies, however, the density of NPY fibers was significantly increased, and that of VIP fibers was unchanged (Fig. 4). Therefore, the changes in the density of VIP and NPY fibers were reversed. The mean density of NPY fibers per unit area was about 1.3 times higher than that of NPY fibers in controls. The mean density of SP and CGRP fibers per unit area was about 0.8 times higher than that of these fibers in controls (Fig. 4). Thus the distribution pattern of peptidergic fibers in the chronically hypercapnic hypoxic carotid body was similar to that in the normoxic carotid body (Fig. 4).

No glomus cells with the immunoreactivity of these four neuropeptides were observed in the normoxic and chronically systemic hypoxic carotid bodies.

In the chronically hypercapnic hypoxic carotid bodies, the density per unit area of NPY immunoreactive nerve fibers was significantly increased, and that of VIP fibers was unchanged. However, in the chronically hypocapnic and isocapnic hypoxic rat carotid bodies, the density per unit area of NPY and VIP fibers was reversed (Kusakabe et al., 1998b, 2000). The difference in the density of NPY and VIP fibers between the hypercapnic and the isocapnic hypoxic carotid bodies may suggest the involvement of a high level of arterial CO_2 tension, although there was no difference in the

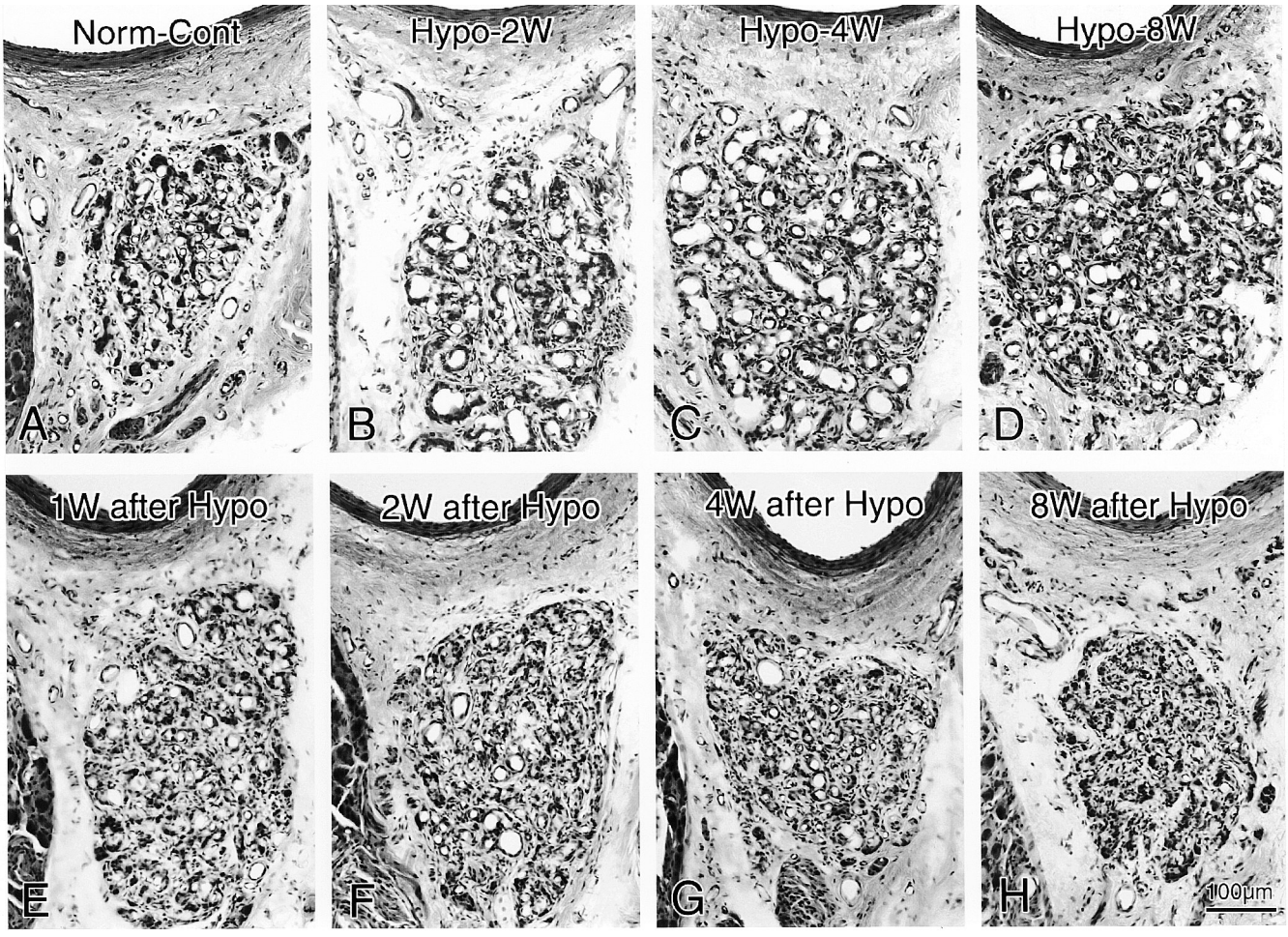

Fig. 5. Hematoxylin-eosin stained sections of a control normoxic carotid body (CB) **(A)**, a carotid body after 2 **(B)**, 4 **(C)**, and 8 weeks **(D)** of hypoxic exposure, and a carotid body 1 **(E)**, 2 **(F)**, 4 (**G)**, and 8 weeks **(H)** after the termination of chronically hypocapnic hypoxia. The hypoxic carotid bodies are enlarged with vascular enlargement in comparison with normoxic control. The carotid bodies after the termination of chronic hypoxia were diminished in size. SCG, superior cervical ganglion. (Kusakabe et al., 2003, 2004b).

density of these fibers between the isocapnic and the hypocapnic carotid bodies. VIP is thought to have a long acting vasodilatory effect (Larson et al., 1976; Heistad, et al., 1980; Wilson et al., 1981). On this basis, we suggested that at least part of the vascular expansion in the chronically hypocapnic and isocapnic hypoxic carotid bodies may be due to the vasodilatory effect of VIP (Kusakabe et al., 1998b). In addition, it has been thought that the vascular enlargement is at least to great extent due to structural changes rather than functional states because there is a true angiogenesis in the hypoxic carotid body. As a result of the vascular enlargement, the blood flow in the chronically hypoxic carotid body is increased. Accordingly we concluded that parenchymal VIP fibers, especially around the blood vessels, are indirectly involved in chemosensory mechanisms by controlling local carotid body circulation (Kusakabe et al., 1998b). In various mammalian vasculature systems, NPY is thought to have a vasoconstrictory effect (Lundberg et al., 1982; Edvinsson et al., 1983a,b; Brain et al., 1985). We speculate that a low percentage of vascular enlargement in the chronically hypercapnic hypoxic carotid body may be due to the vasoconstrictory effect of the increased NPY resulting from hypercapnia. However, this speculation may only be true of the chronically hypoxic rat carotid body. To make this clear, it is necessary to perform further morphological studies in the vasculature of various organs in hypercapnic hypoxic animals.

Some authors have reported an involvement of SP- and CGRP-containing fibers 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; Finley et al., 1992). Other authors have shown a dose-related increase in chemosensory discharge after SP injection into the carotid body (McQueen, 1980; Cragg et al., 1994). On the other hand, some authors have suggested the possibility that the efferent component of the glossopharyngeal nerve possesses vasodilatory SP and CGRP (Hallberg and Pernow, 1975; Samnegard et al., 1978; Edvinsson et al., 1981; Edvinsson and Uddman, 1982; Brain et al., 1985) in addition to its sensory role. In the chronically hypercapnic hypoxic carotid body, the density of SP and CGRP immunoreactive fibers was significantly decreased, although that of SP and CGRP fibers in the chronically hypocapnic and isocapnic hypoxic carotid bodies was unchanged (Kusakabe et al., 1998b, 2000). This difference in the density of SP and CGRP may also be dependent on the high level of arterial CO_2 tension in chronically hypercapnic hypoxia.

Thus, the different levels of CO_2 tension change the peptidergic innervation in the carotid body during chronically hypoxic exposure. Altered peptidergic innervation may regulate vascular tone in the chronically hypoxic carotid body.

Changes in the rat carotid body during the course of hypoxic adaptation and during the course of recovery

As mentioned in the introduction, most authors use the term "chronic hypoxia" in their works, regardless of varying periods of experimental hypoxic exposure, from 1-2 weeks to 2-3 months. To avoid confusion, it is necessary to demonstrate an identical survey in the carotid body after different periods of hypoxic exposure. On the other hand, most previous studies on the hypoxic carotid bodies have referred to the changes after hypoxic exposure. It is also meaningful for high altitude exercise to make clear the morphological changes in the chemoreceptor organs during deacclimatization after chronic hypoxia is terminated. To evaluate the different levels of hypoxic exposure, the morphological changes in the rat carotid body, especially those in the vasculature, during each of the three periods of hypoxia

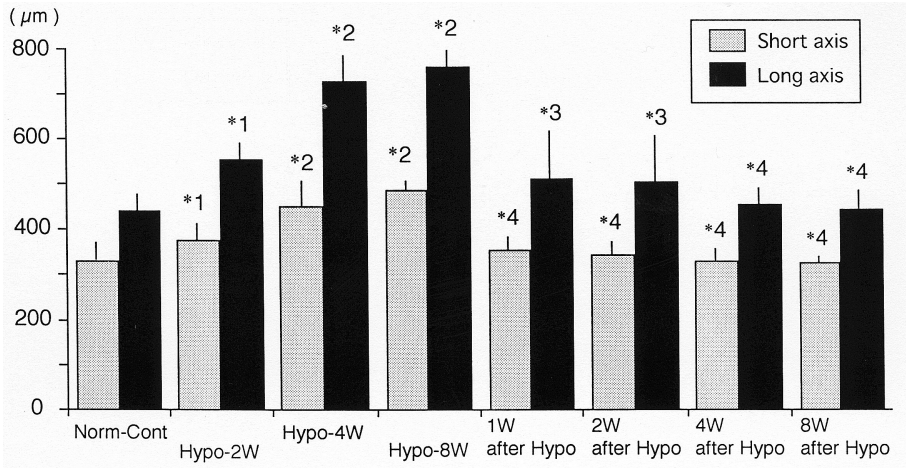

Fig. 6. Histogram comparing the short and long axes of normoxic control carotid bodies, those after 2, 4, and 8 weeks of hypocapnic hypoxic exposure, and those 1, 2, 4, and 8 weeks after the termination of hypoxia. *1 $p<0.01$, and *2 $p<0.005$ in comparison with the normoxic control column. *3 $p<0.01$, and *4 $p<0.005$ in comparison with the Hypo-8W column. (Kusakabe et al., 2003, 2004b).

were recently examined (Kusakabe et al., 2003). Those in the carotid bodies four periods after the termination of chronic hypoxia were also examined to evaluate the different levels of recovery (Kusakabe et al., 2004b).

The carotid bodies of rats exposed to hypoxia for 2, 4, and 8 weeks were found to be enlarged several fold in comparison with those of normoxic control rats. The rate of enlargement was different for the carotid bodies exposed for three different periods (Fig. 5A-D). The mean short axis of the carotid bodies of the rats exposed to hypoxia for 2, 4, and 8 weeks was 1.2, 1.3, and 1.5 times longer than in normoxic controls, respectively (Fig. 6). The mean long axis was 1.3, 1.6, and 1.7 times longer than in normoxic controls, respectively (Fig. 6). With a prolonged hypoxic exposure, the percentage of blood vessels with relatively wide lumens, more than 21 µm, increased, and the percentage of vessels with relatively narrow lumens, less than 5-10 µm, decreased (Fig. 7). Thus, the enlargement of the hypoxic carotid bodies was mainly due to vascular dilation as suggested by Blessing and Wolff (1973), and Laidler and Kay (1975a,b). Pequignot and Hellström (1983) reported that vascular dilation is already evident in the rat carotid bodies after 1 week of exposure to hypoxia. It seems likely that the enlargement of the carotid bodies with vascular expansion begins soon after the start of hypoxic exposure. As far as enlargement of the carotid bodies is concerned, the use of the term "chronic hypoxia" has little meaning as a general expression regardless of the duration of hypoxic exposure.

The carotid bodies 1 week after the termination of chronic hypoxia were significantly diminished in size in comparison with the carotid bodies of rats exposed to hypoxia for 8 weeks, and the carotid bodies 8 weeks after the termination of hypoxia were similar to the normoxic controls in size (Fig. 5E-H). This indicates that recovery in the carotid bodies had already started relatively early, i.e., 1 week after the termination of chronic hypoxia, and complete recovery occurred by 4-8

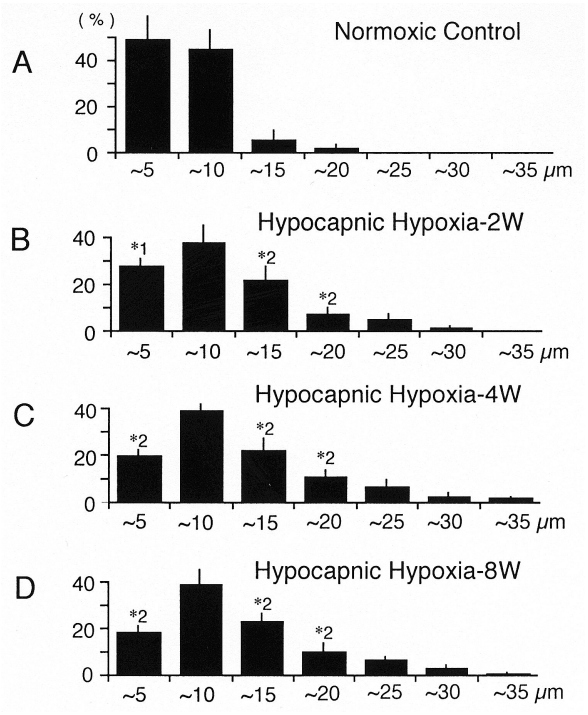

Fig. 7. Histograms representing the percentage of blood vessels of seven ranges of diameter in normoxic control carotid bodies **(A)** and those after 2 **(B)**, 4 **(C)**, and 8 weeks **(D)** of hypoxic exposure (Kusakabe et al., 2003).

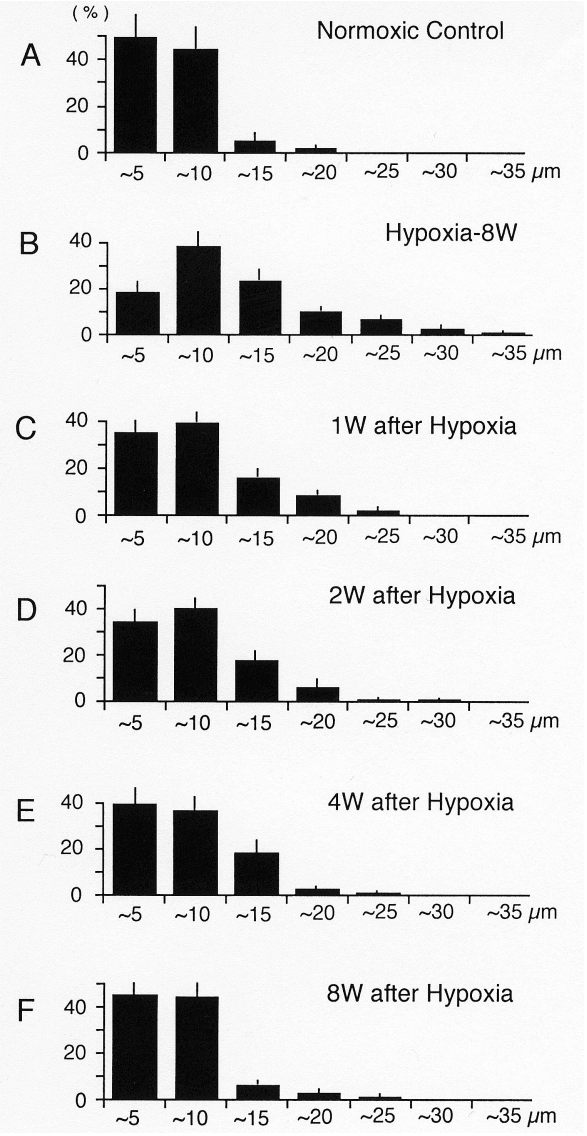

Fig. 8. Histograms representing the percentage of blood vessels of seven ranges of diameter in normoxic control carotid bodies **(A)**, those after 8 weeks of hypoxic exposure **(B)**, and those 1 **(C)**, 2 **(D)**, 4 **(E)**, and 8 **(F)** weeks after the termination of hypoxia (Kusakabe et al., 2004b).

weeks after the termination of hypoxia (Fig. 6). In the carotid bodies 1, 2, and 4 weeks after the termination of chronic hypoxia, the percentage of large blood vessels with diameters greater than 16 µm decreased, and that of small vessels with diameters less than 10 µm increased (Fig. 8A-E). In the carotid bodies 8 weeks after the termination of hypoxia, the percentage of large vessels with diameters greater than 16 µm further decreased to 8.2%, and that of diameters less than 5 µm further increased to 89.6%, which was a condition similar to that in normoxic control carotid bodies (Fig. 8F). As stated above, the enlargement of chronically hypoxic carotid bodies is mainly due to vascular dilation (Blessing and Wolff, 1973; Laidler and Kay, 1975; Pequignot and Hellström, 1984). Naturally, shrinking of the carotid bodies after the termination of hypoxia is also due to vascular contraction. In the course of recovery, vascular contraction is also evident in the carotid bodies 1 week after the termination of hypoxia. Thus, it seems likely that shrinking of the carotid bodies with vascular contraction begins soon after the termination of hypoxic exposure.

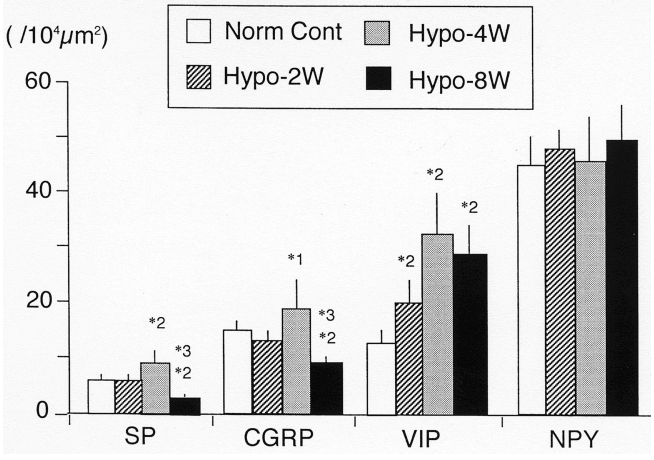

Fig. 9. Histogram comparing the density of varicosities per unit area in normoxic control carotid bodies (Norm Cont), and those after 2, 4, and 8 weeks of hypoxic exposure. *1 p<0.01, and *2 p<0.005 in comparison with the control column, and *3 p<0.005 in comparison with the 4-week column (Kusakabe et al., 2003).

Peptidergic innervation in the rat carotid body during the course of hypoxic adaptation and during the course of recovery

As stated above, the changes in the peptidergic innervation 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. Altered peptidergic innervation in the chronic hypoxic carotid bodies may show a completely acclimatized state. To show the precise distribution of the different levels of hypoxic exposure, the changes in the peptidergic innervation in the rat carotid body during each of the three periods of hypoxia were recently examined (Kusakabe et al., 2003). Those in the carotid bodies four periods after the termination of chronic hypoxia were also examined (Kusakabe et al., 2004).

SP, CGRP, VIP, and NPY immunoreactive fibers appeared as thin processes with many varicosities, and most of them were associated with the blood vessels, as mentioned in the former section. 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 (Fig. 9), and in those four periods after the termination of hypoxia (Fig. 10). Mean density per unit area of SP and CGRP immunoreactive fibers was transiently high in the carotid bodies after 4

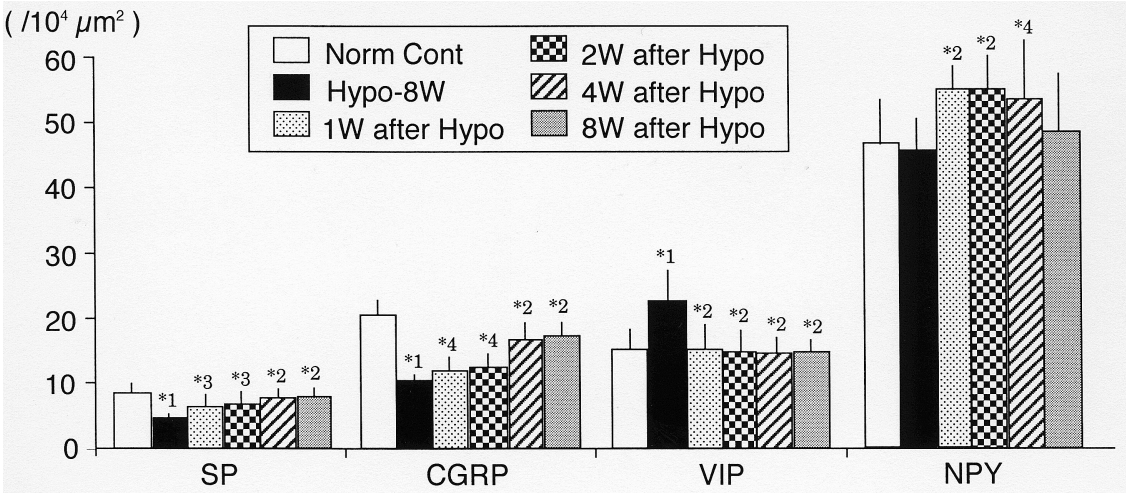

Fig. 10. Histogram comparing the density of varicosities per unit area in normoxic control carotid bodies (Norm Cont), those after 8 weeks hypoxic exposure, and those 1, 2, 4,and 8 weeks after the termination of hypoxia. *1 p<0.005 in comparison with the normoxic control column. *2 p<0.005, *3 p<0.01, and *4 p<0.05 in comparison with the Hypoxia-8W column. (Kusakabe et al., 2004b).

weeks of hypoxic exposure, and decreased significantly to nearly or under 50% after 8 weeks of hypoxic exposure (Fig. 9). Density of VIP immunoreactive fibers increased significantly in all periods of hypoxic exposure, and was especially high after 4 weeks of hypoxic exposure (Fig. 9). Density of NPY immunoreactive fibers was unchanged in the carotid bodies during hypoxic exposure (Fig. 9). SP and CGRP fibers in the carotid bodies after 4 weeks of hypoxic exposure may be involved in both chemosensory and vascular dilatory systems, although we previously reported that SP and CGRP fibers in carotid bodies after chronic hypoxic exposure may not be involved in chemosensory mechanisms because the density of immunoreactive fibers at this stage decreases to under 50% (Kusakabe et al., 1998b, 2000). It seems likely that VIP is more effective in the carotid bodies after 4 weeks of exposure than in carotid bodies after longer exposure, and that physiological involvement of NPY fibers is invariable from short to prolonged hypoxic exposure. Thus, the characteristic changes in the peptidergic innervation suggest that the role of peptidergic 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.

After the termination of hypoxia, the most striking feature of the peptidergic innervation is immediately increased density of NPY fibers and immediately decreased density of VIP fibers in the carotid bodies 1- 4 weeks after the termination of chronic hypoxia (Fig. 10). We previously speculated that at least part of the vascular dilation in the chronically hypoxic rat 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 (Kusakabe et al., 1998b). In various mammalian vasculature systems, NPY is thought to have a vasoconstrictory effect (Lundberg et al., 1982; Edvinsson et al., 1983; Brain et al., 1985). The percentage of blood vessels with relatively narrow lumens decreased in the carotid bodies 1 week after the termination of chronic hypoxia. It seems likely that shrinking of the carotid body with vascular contraction is caused by the increased density of vasoconstrictive NPY. At least part of the vascular constriction in the carotid bodies in recovery stages may depend on the vasoconstrictory effect of NPY. Thus, the morphological changes during recovery stages may be under the control of peptidergic innervation.

Concluding remarks

Our recent projects on the carotid bodies of the rats exposed to systemic hypoxia clarified the relation between some morphological changes and the different levels of arterial CO_2 tension. The enlarged hypoxic carotid bodies contained a number of blood vessels whose diameter was larger than those in the normoxic carotid bodies, and the percentage of vascular enlargement in the hypercapnic hypoxic carotid body was small in comparison with that in the hypocapnic and isocapnic hypoxic carotid bodies. This characteristic vascular arrangement was under the control of altered peptidergic innervation during chronically hypoxic exposure. As mentioned above, the vascular constriction in the hypercapnic hypoxic rat carotid body may be restricted to the organs in hypoxic conditions. More recently, we compared the morphological changes and those in the peptidergic innervation between the carotid bodies of the rats exposed to hypercapnic hypoxia and those exposed to normoxic hypercapnia (Kusakabe et al., 2005). In brief, we consider that CO_2 may have some additive effects on the chemoreceptor organs in hypoxic conditions, because high CO_2 tension in normoxic conditions does not cause morphological changes in the rat carotid body.

Through the observations during the course of hypoxic adaptation and during the course of recovery, the enlargement of the carotid bodies with vascular expansion and the shrinking of the carotid bodies with vascular contraction begin soon after the start of hypoxic exposure and soon after the termination of hypoxic exposure. All carotid bodies after 2, 4, and 8 weeks of hypoxic exposure were enlarged with vascular expansion although the amount of enlargement was different for each. As far as macroscopic findings such as the enlargement of the carotid body is concerned, the use of the term "chronic hypoxia" has little meaning as a general expression regardless of the duration of hypoxic exposure. In the case of microscopic findings such as the changes in the peptidergic innervation, it is necessary to be careful about the use of the term "chronic hypoxia", because some peptidergic fibers showed a transiently increased density during the course of hypoxic exposure.

Our recent studies may provide a morphological standard for further studies of the hypoxic carotid bodies. Finally we expect a wide application of this morphological standard when comparing with a number of findings under various hypoxic conditions.

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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. (1983a). Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. Neurosci. Lett. 43, 79-84.

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

Fenstermacher J.D. and Rapoport S.I. (1984). Blood-brain barrier. In: Handbook of Physiology. Volume IV. Microcirculation, Part 2. Waverly Press. Baltimore. pp 969-1000.

Finley J.C.W., Polak J. and Katz D.M. (1992). Transmitter diversity in carotid body afferent neurons - Dopaminergic and peptidergic phenotypes. Neuroscience 52, 973-987.

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

Hayashida Y., Hirakawa H., Nakamura T. and Maeda M. (1996). Chemoreceptors in autonomic responses to hypoxia in conscious rats. Adv. Exp. Med. Biol. 410, 439-442.

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.

Heistad D.D., Marcus M.I., Said S.I. and Gross P.M. (1980). Effect of acetylcholine and vasoactive intestinal peptide on cerebral blood flow. Am. J. Physiol. 238, H73-H80.

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.

Kondo H. and Yamamoto M. (1988). Occurrence, ontogeny, ultrastructure and some plasticity of CGRP (calcitonin gene-related peptide)-immunoreactive nerves in the carotid body of rats. Brain Res. 473, 283-293.

Kondo H., Kuramoto H. and Fujita T. (1986). Neuropeptide tyrosine-like immunoreactive nerve fibers in the carotid body chemoreceptor of rats. Brain Res. 372, 353-356.

Koehler R.C., McDonald B.W. and Krasney J.A. (1980). Influence of CO_2 on cardiovascular response to hypoxia in conscious dogs. Am. J. Physiol. 239, H545-H558.

Kusakabe T. (2002) Carotid labyrinth of amphibians. Microsc. Res. Tech. 59, 207-226.

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., Kawakami T. and Takenaka T. (1993a). Distribution of FMRFamide-immunoreactive nerve fibers in the carotid labyrinth of the bullfrog, Rana catesbeiana in corresponding differential interference-contrast (Nomarski) images. Arch. Histol. Cytol. 56, 277-282.

Kusakabe T., Powell F.L. and Ellisman M.H. (1993b). 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., Kawakami T., Tanabe Y., Fujii S., Bandou Y. and Takenaka T. (1993c). Coexistence of substance P and calcitonin gene-related peptide in the nerve fibers of the carotid labyrinth of the bullfrog, Rana catesbeiana. Brain Res. 603, 153-156.

Kusakabe T., Kawakami T. and Takenaka T. (1994a). Coexistence of substance P, neuropeptide Y, VIP, and CGRP in the nerve fibers of the carotid labyrinth of the bullfrog, Rana catesbeiana: a double labelling immunofluorescence study in combination with alternate consecutive sections. Cell Tissue Res. 276, 91-97.

Kusakabe T., Kawakami T., Tanabe Y., Fujii S., Bandou Y. and Takenaka T. (1994b). Coexistence of substance P and calcitonin gene-related peptide in the nerve fibers of the carotid labyrinth of the bullfrog, Rana catesbeiana. Brain Res. 603, 153-156.

Kusakabe T., Kawakami T. and Takenaka T. (1995a). Peptidergic innervation in the amphibian carotid labyrinth. Histol. Histopathol. 10, 185-202.

Kusakabe T., Kawakami T., Ono M., Hori H. and Takenaka T. (1995b). Localization of galanin-immunoreactive nerve fibers in the carotid labyrinth of the bullfrog, Rana catesbeiana: comparison with substance P-immunoreactive fibers. Cell Tissue Res. 281, 63-67.

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.

Kusakabe T., Hirakawa H., Oikawa S., Matsuda H., Kawakami T., Takenaka T. and Hayashida Y. (2003). Peptidergic innervation in the rat carotid body after 2, 4, and 8 weeks of hypocapnic hypoxic exposure, Histol. Histopathol. 18, 409-418.

Kusakabe T., Matsuda H. and Hayashida Y. (2004a). Rat carotid bodies

in systemic hypoxia: Involvement of arterial CO_2 tension in morphological; changes. Adv. Exp. Med. Biol. 475, 793-799.536, 611-617

Kusakabe T., Hirakawa H., Matsuda H., Kawakami T., Takenaka T. and Hayashida Y. (2004b). Morphological changes in the rat carotid body 1, 2, 4, and 8 weeks after the termination of chronically hypocapnic hypoxia, Histol. Histopathol. 19, 1133-1140.

Kusakabe T., Matsuda H. and Hayashida Y. (2005). Effect of carbon dioxide on the structure of the carotid body: a comparison between normoxic and hypoxic conditions. Adv. Exp. Med. Biol. (in press).

Laidler 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.

Laidler 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.

Laidler 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.

Larson L.I., Edvinsson L., Fahrenkrug R., Håkanson R., Owman C.H., Schaffalitzky de Muckadell O.B., Sundler F. (1976). Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves. Brain Res. 113, 153-156.

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

Lundberg J.M., Hökfelt T., Fahrenkrug J., Nilsson G. and Terenius L. (1979). Peptides in the carotid body (glomus caroticum): VIP-, enkephalin- and substance P-like immunoreactivity. Acta Physiol. Scand. 107, 279-281.

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., Traystman R.J. (1981). Vasoactive intestinal polypeptide and canine cerebral circulation. Circ. Res. 48, 138-148.

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