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Review

Structural and neurochemical changes in the maturation of the carotid body☆

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ABSTRACT

Functional maturation of the carotid body in the postnatal period relies partly on structural and neurochemical changes, which are reviewed here. Structural changes include changes in cytological composition, and increases in glomic tissue volume, dense-cored granules of type I cells, synapses of type I cells with type II cells and afferent nerve fibres. Vascular volume also increases, but in the same proportion as extravascular volume. During maturation, the carotid body also shows higher density and hypoxic sensitivity of K^+-channels and an increased hypoxic $[Ca^{2+}]_i$ response. Modulation of content and release of catecholamine occurs, together with decreased expression of tyrosine hydroxylase and dopamine β-hydroxylase and increased expression of choline acetyltransferase. Expression of dopamine 2 receptor and nicotinic α3 and α7 receptor subunits increases, and muscarinic M1 receptor protein, nicotinic α4 and β2 receptor subunits and adenosine receptor 1 decrease. Maturation of the carotid body may also be explained with reference to the developmentally regulated expression of trophic factors and their receptors.

1. Introduction

The carotid body, an ellipsoid tissue mass located at the carotid bifurcation, is the main peripheral arterial chemoreceptor. It stimulates the ventilatory response, acting on central respiratory centres, in response to hypoxia, hypercapnia or reduced blood pH. From a structural point of view, it is composed of lobules containing type I cells, positive for tyrosine hydroxylase (TH) and distinguished into light, dark and pyknotic/progenitor, and type II cells, positive for glial fibrillary acidic protein (GFAP). Type I cells (also called chief or glomus cells) are roundish in shape and are considered the true chemoreceptor elements. Many neurotransmitters (e.g., dopamine, noradrenaline, adrenaline, acetylcholine, serotonin, adenosine) and peptide neuromodulators (e.g., enkephalins, neuropeptide Y, calcitonin gene-related peptide, galanin, endothelins, bombesin, adrenomedullin, kisspeptins, leptin) have been identified in type I cells. They are mainly contained in dense-cored cytoplasmic granules and are released in response to the above different kinds of stimuli. Type II cells (also called sustentacular cells) are fusiform and envelop clusters of type I cells. They mainly play a supportive role, but may also behave as stem cell precursors for type I cells (Pardal et al., 2007). Type II cells have also been demonstrated to show a transient $[Ca^{2+}]_i$ rise in response to ATP, angiotensin II and muscarine, and have been suggested to co-ordinate chemosensory transduction through interactions with nerve endings, type I cells and blood vessels (Tse et al., 2012). Neurotransmitters and neuromodulators released by type I cells act on the afferent endings of the glossopharyngeal nerve arising from the petrosal ganglion. The carotid body also shows sensory innervation from jugular and nodose ganglia, post-ganglionic sympathetic nerve fibres from the superior cervical ganglion, and preganglionic parasympathetic and sympathetic fibres reaching ganglion cells in the carotid body. Efferent parasympathetic and sympathetic innervation of the carotid body plays a pivotal role in modulation of blood flow.

Animal and human studies indicate that maturation of the peripheral arterial chemoreceptors is protracted in the postnatal period. At birth, there is an initial silencing of peripheral arterial chemoreceptors, and their activity is not considered necessary in order to establish rhythmic breathing (Jansen et al., 1981). In the following postnatal period, a gradual increase in hypoxic chemosensitivity develops, with change in the hypoxic threshold (Blanco et al., 1984; Gauda et al., 2004a).

Many studies in the literature have tried to identify perinatal and postnatal changes in the carotid body which may at least partially explain the functional maturation of the carotid body. Attention mainly focused on structural aspects, such as morphometric parameters (volume, number of cells, proportion of different cell types, vascular compartment) and on changes in the expression of factors involved in O_2 sensing, such as membrane channels, neurotransmitters/neuromodulators, growth factors and receptors. The

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Table 1
Main structural changes in carotid body maturation.

Parameter	Variation	Species	Period	Refs.
Volume or section areas	↑	Human	Birth → 4.5 y	Dinsdale et al., 1977
	↑	Human	First year of life	Lack et al., 1986
	↑	Cat	PN1 → adult	Clarke and Daly, 1985, Clarke et al., 1990
	≈	Rabbit	PN1 → 12-wk	Bolle et al., 2000
Percentage volume of small vessels	≈	Cat	Foetus → newborn → adult	Clarke et al., 1990
Percentage volume of large vessels	↑	Lamb	Foetus → newborn	Moore et al., 1991
Blood velocity	↓	Lamb	Foetus → 1-wk	Acker et al., 1991
Dense-core granules in type I cells	↑	Rat	First postnatal week	von Dalnok and Menssen, 1986
Type I–type II synapses	↑	Rat	Newborn	Kondo and Iwasa, 1996
Type I–afferent fibres synapses	↑	Rat	Perinatal period	Kondo, 1976
Afferent nerve endings	↑	Rabbit	PN1 → 12-wk	Bolle et al., 2000
Efferent nerve endings	↓	Rabbit	PN1 → 12-wk	Bolle et al., 2000

mo, month; PN, postnatal day; y, year; wk, week.

aim of the present paper is to provide a brief review of the structural and neurochemical changes occurring in the carotid body during perinatal development.

2. Structural changes

2.1. Volume of the carotid body

The main structural changes during maturation are listed in Table 1. For instance, the total volume of the infant carotid body has been reported to increase from 0.3 mm^3 at birth to 1.2 mm^3 at 4.5 years. The volume of glomic tissue also increases in the same age range from 0.08 mm^3 to 0.47 mm^3 (Dinsdale et al., 1977). Gradual increments in combined weights and the total and functional surface areas of carotid bodies have also been reported by Lack et al. (1986) during the first year of life in infants. In cats, significant increases in the volume of the carotid body have been reported from foetuses (0.048 mm^3) to newborns (0.082 or 0.072 mm^3) and from newborns to adults (0.247 or 0.184 mm^3) (Clarke and Daly, 1985; Clarke et al., 1990). These values are consistent with our personal experience on human and rat samples (Figs. 1 and 2). Conversely, no significant changes in the sectional area of glomus cell groups have been observed in rabbits aged from 1 day to 12 weeks (Bolle et al., 2000). Apart from the above data about the volume of the carotid body in toto and its glomic component, further analyses could address the developmental aspects of interlobular and intralobular connective tissue, these components being quite easy to evaluate morphometrically on azan Mallory- or Syrius red-stained sections (e.g., Porzionato et al., 2005).

2.2. The carotid body as a neurogenic niche

Some authors have occasionally found mitoses in the carotid body of newborn kittens (Clarke and Daly, 1985), rats (von Dalnok and Menssen, 1986) and human infants (Heath et al., 1990). Type I cells from newborn rats have been found to divide in culture (Fishman and Schaffner, 1984; Nurse and Fearon, 2002) and Wang and Bisgard (2005) identified bromodeoxyuridine-labelled type I cells in newborn rats, providing evidence of proliferation of type I cells in the postnatal period. The same authors did not report type I cell divisions in rat carotid bodies six to eight weeks after birth. Proliferation of type II cells has also been described in newborn rats, although less frequently than type I cells (Wang and Bisgard, 2005).

The above findings, however, should be reconsidered in the light of later studies identifying the carotid body as a neurogenic niche. Pardal et al. (2007) discovered that GFAP+ type II cells may be activated in response to hypoxia, proliferate, and differentiate into TH+ type I cells, through an intermediate step involving GFAP− nestin+ progenitor cells. Type II cells were identified as carotid body stem cells. The above authors did not exclude the possibility that in newborn animals TH+ cells retain their mitogenic potential, but suggest that in adults BrdU+ TH+ cells derive from stem cells. The above study was mainly addressed to structural adaptations of the carotid body to hypoxia (10% O_2 for 21 days) and renormoxia (exposure to 21% O_2 for 1 month after hypoxic exposure), but further studies must evaluate the role of type II/stem cells in normal development/maturation of the carotid body and in adaptative responses to other environmental stimuli. Obviously, it will also be essential to confirm the above findings on human material.

2.3. Cell populations of the carotid body

During maturation of the human carotid body, significant changes have also been reported in the various cell populations. Hervonen and Korkala (1972) described the structure of the carotid body in human foetuses at 14–15 weeks of gestation. They found surprisingly mature microscopic anatomy, showing type I cells with dense-cored vesicles, type II cells enveloping chief cells, and rich innervation. Other authors reported different descriptions in later gestational periods. In foetuses from 23 to 30 weeks of gestational age, the carotid body has been reported to show ill-defined lobules of primitive cells with oval/round nuclei and faintly eosinophilic cytoplasm. Progenitor, dark and light variants of chief cells were less numerous; well-defined groups of elongated sustentacular cells were not yet present. After this gestational age, sustentacular cells and the three variants of chief cells were reported to be clearly visible, and primitive cells became rarer and were no longer visible after birth. Dark cells then increased in number in the first months and reached adult levels at about 5 months. The number of light cells also increased from 11% at birth to around 30% at the age of 3.5 months (Heath et al., 1990). Fig. 1 shows structural changes in the human carotid body from the foetal to adult period. Pavai et al. (2005) did not report significant changes in the number of dark and light cells between a group of non-Sudden Infant Death Syndrome (SIDS) infants (mean age: 5.5 months, age range: 1–12 months) and adults. However, from a methodological point of view it must be recalled that some criticism has arisen about the significance of the different variants of the chief cells, the percentage of progenitor/pycnotic cells being found to increase with postmortem time (Seker et al., 1994). More reliable data about the relative proportions of type I and type II cell populations may also come from studies with double immunohistochemical and immunofluorescence methods (e.g., Porzionato et al., 2005).