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PRENATAL DEVELOPMENT OF RESPIRATORY CHEMORECEPTORS IN ENDOTHERMIC VERTEBRATES

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Abstract

Respiratory chemoreceptors are neurons that detect PCO₂, PO₂, and/or pH in body fluids and provide sensory feedback for the control of breathing. They play a critical role in coupling pulmonary ventilation to metabolic demand in endothermic vertebrates. During birth in mammals and hatching in birds, the state change from placental or chorioallantoic gas exchange to pulmonary respiration makes acute demands on the neonatal lungs and ventilatory control system, including the respiratory chemoreceptors. Here we review the literature on prenatal development of carotid body chemoreceptors, central chemoreceptors, and airway chemoreceptors, with emphasis on the histology, histochemistry, and neurophysiology of chemosensory cells or their afferents, and their physiological genomics if known. In general, respiratory chemoreceptors develop prenatally and are functional but immature at birth or hatching. Each type of respiratory chemoreceptor has a unique prenatal developmental timecourse, and all studied to date require a period of postnatal maturation to express the full adult response.

Keywords

carotid; central; chemoreceptor; mammal; bird; intrapulmonary; embryo; fetus

1. Introduction

Respiratory chemoreceptors, including O₂–, CO₂–, and pH-sensitive carotid bodies, and CO₂–sensitive central and airway chemoreceptors, provide critical sensory feedback for ventilatory control and metabolic homeostasis in endothermic vertebrates. At birth, the conversion from placental or chorioallantoic gas exchange to pulmonary gas exchange makes rapid demands on the lungs and ventilatory control system (Mortola, 2011). While this abrupt transition in the respiratory apparatus is critical, even necessary, for proper maturation of several respiratory structures, the neuromuscular and gas exchanging system must be sufficiently developed to meet the immediate metabolic needs of the newborn. As will be summarized here, current evidence indicates that respiratory chemoreceptors are present at birth, but require post-natal maturation to express the full adult response. Abnormal genetic or environmental factors can impact prenatal and/or postnatal chemoreceptor development with debilitating or fatal consequences, including congenital

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central hypoventilation syndrome (CCHS, Ondine's curse), sudden infant death syndrome (SIDS), and lifelong deficits in reflex responses to respiratory gases.

The following review will consider the prenatal development of carotid body chemoreceptors and central chemoreceptors, in birds and mammals, as well as avian intrapulmonary chemoreceptors. The focus is on functional characteristics of the chemoreceptors or their afferents, including, where known, their histology, developmental genetics, biochemistry, innervation, and electrophysiology. Review of the development of integrated motor responses to O₂, CO₂ and/or pH stimuli in the embryo will be considered in another paper in this volume (Mortola, 2011).

2. Prenatal Development of Carotid Body Chemoreceptors

2.1 Background

Carotid bodies are complex, neural crest derived organs found at the bifurcations of the left and right common carotid arteries in mammals and birds (Pearse et al., 1973). They are the primary respiratory chemoreceptors for arterial PO₂, but are also sensitive to arterial PCO₂ and pH. Adult carotid bodies contain characteristic structural elements, including: glomus (type I) cells, the presumed chemosensors, which are depolarized by hypoxia, hypercapnia, and acidosis, and have vesicles that contain dopamine, acetylcholine, substance P, and other neurotransmitters; afferent fibers which receive synaptic input from the glomus cells, have axons carried in the carotid sinus nerve, somata in the petrosal ganglion (nodose in birds), and transmit action potentials from the CB to the solitary tract nuclei in the brainstem; efferent nerve fibers from the superior cervical ganglia which synapse with glomus cells and modulate excitability to hypoxia; and sustentacular (Type II) cells of the carotid body which are glial-like and envelop the glomus cells and nerve fibers, but are not excitable. Blood vessels richly perfuse the carotid body with arterial blood, creating the "glomerulus"-like vascularization characteristic of the organ.

2.2 Prenatal Carotid Body Histology

The histology of the carotid body in midterm human fetuses (ca. 14–15 weeks) has been described by Hervonen and Korkala (1972) as "*surprisingly mature*". Glomus (Type I) cells were plentiful in midterm fetuses and possessed dense-cored vesicles with strong formaldehyde-induced fluorescence characteristic of monamines. Evidence of exocytosis of vesicle contents was captured in some micrographs. Type II (sustentacular cells) closely enveloped glomus cells, blood capillaries, and nerve fibers that innervated the glomus cells. Nerve fibers richly innervated glomus cells, and their terminals contained both agranular and granular vesicles. Blood capillaries filled about 10–55% of the carotid body organ volume (Hervonen et al., 1972). Thus, even at midterm, human fetal carotid bodies appear to have a basic structure similar to adult carotid bodies. Relatively mature histological development of the carotid bodies is also seen in fetal sheep and fetal rats (reviewed by Hanson, 1986).

Nevertheless, prenatal development does not produce adult-type carotid bodies at birth. Postnatal changes occur during the first few weeks after birth, which produce a significant increase in carotid body hypoxic responsiveness to adult-like levels (reviewed by Gauda et al., 2009). Post-natal maturation includes increases in: hypoxic inhibition of background leakage K currents in glomus cells (Wasicko et al., 1999), the proportion of glomus cell K⁺ current that is inhibited by hypoxia (Hatton et al., 1997), the glomus cell intracellular calcium responses to hypoxia (Wasicko et al., 1999), the hypoxia-induced secretion of catecholamines from glomus cells (Donnelly, 2005), the magnitude and dynamic sensitivity of carotid sinus afferent discharge to hypoxia (Kholwadwala et al., 1992), and the innervation density of the glomus cells by nodose-petrosal ganglion nerve fibers (Donnelly, 2005; Tolosa et al., 2005).

2.3 Prenatal Development of Carotid Body Chemosensitivity

The electrical responses of fetal carotid body afferents, measured extracellularly from axons carried in the carotid sinus nerves, were first reported in fetal sheep. Biscoe et al. (1969) found that the discharge of carotid body chemoreceptor fibers in late term lamb fetuses (120–147 days) was insensitive to arterial PO_2 and sodium cyanide, but was stimulated by clamping the umbilical cord or by stimulating the cervical sympathetic nerves (perhaps mimicking some of the events associated with birth). In a related study, Biscoe and Purves (1967) reported that in new-born lambs (0–5 days old), carotid body chemoreceptor discharge response to arterial PO_2 was fully mature (adult-like) and did not change postnatally through day 5.

Subsequent studies using similar preparations could not reproduce the results of Biscoe and Purves (1967) and Biscoe et al. (1969). In the intervening years, experiments on the development of carotid body afferent activity have revealed both prenatal and postnatal maturation processes in carotid chemoreceptor function (reviewed by Hanson, 1986; Mulligan 1991; Gauda et al., 2009, and summarized below).

Blanco et al. (1984) measured carotid body chemoreceptor afferent activity from sinus nerve filaments in anesthetized, exteriorized fetal lambs, gestational age 90–143 days, and from newborn lambs. They found that fetal carotid chemoreceptor fibers discharged at about 5 Hz at 25 mmHg PaO_2 , and increased discharge as PaO_2 was reduced. Thus, fetal carotid body chemoreceptor fibers were not only active *in-utero*, they were responsive to hypoxia. However, the hypoxic threshold (PO_2) required for stimulation was lower than in adults (the fetal chemoreceptor PaO_2 sensitivity curve was left-shifted to a lower PO_2 range). Fetal lamb chemoreceptor fibers increased discharge rapidly to intra-arterial infusion of CO_2 saturated saline but not to infusion of pH 7.4 saline or fetal arterial blood; they increased discharge when the umbilical cord was ligated and PaO_2 fell, and decreased discharge when the fetal lungs were artificially ventilated and PaO_2 rose.

In newborn lambs delivered on gestational days 135–146 and breathing air, Blanco et al. (1984) could find no spontaneous carotid body chemoreceptor activity at all, in contrast to Biscoe and Purves (1967) who found adult-like chemoreceptor discharge at birth. Blanco et al. (1984) found that chemoreceptor activity increased postnatally and was detectable in most lambs at ≥ 2 days of age, and the response to isocapnic hypoxia challenge increased significantly by age 3 days. They concluded that “...the arterial chemoreceptors are active and responsive in the fetus, but quiescent in the lamb on the day of birth when PaO_2 has risen. The hypoxic sensitivity of the chemoreceptors is reset to the adult range over the next few days”. Resetting increasing of the carotid body chemoreceptor response from the low PaO_2 threshold characteristic of fetal life, to a higher PaO_2 threshold characteristic of air breathing and adult life has been subsequently reported in kittens (Hanson 1989) and piglets (Mulligan, 1991), and takes about 1–2 weeks (Mulligan, 1991, Gauda et al., 2009). If the abrupt increase in PaO_2 is prevented by providing hypoxic air for the newborn to breathe, resetting does not occur and the hypoxic sensitivity defect persists into adulthood (Hanson, 1989). Interestingly, providing newborns with hyperoxic inspired gas similarly interferes with resetting (reviewed by Bavis et al., 2008).

To summarize, the basic structural elements of the carotid body chemoreceptors develop prenatally, but fetal carotid bodies have a lower PO_2 threshold presumably due to fetal hypoxemia accompanying placental gas exchange. The carotid bodies are still immature at birth, and must undergo a resetting to a higher PO_2 threshold and further maturation of PO_2 sensitivity during the post-natal period. Resetting and maturation seems to be triggered by the rise in arterial PO_2 to normoxic levels when pulmonary gas exchange replaces placental gas exchange at birth, which transiently silences carotid body responses for several days.

This is a critical time window for initiating normal maturation of carotid body chemosensitivity toward adult levels, which takes several weeks in animal models studied (Gauda et al., 2009).

3. Prenatal Development of Central Chemoreceptors

3.1 Background

Dawes (1984) reported that fetal breathing movements (FBM) can be detected in sheep at 40 days of gestation (term is about 147 days). Near term FBM in sheep are typically episodic and decreased by hypoxia, while breathing at birth is continuous. When episodically present, fetal breathing becomes deeper and more regular with hypercapnia. Similar observations have been made in human fetuses and neonates (reviewed by Darnall, 2010). The hypercapnic ventilatory response (HCVR) is present at birth indicating prenatal development of neural substrates for CO₂ chemoreception. But like the hypoxic ventilatory response, the HCVR continues to mature after birth and significantly increases in magnitude during the postnatal period toward adult levels, reached at about P20 in rats (Stunden et al., 2001; Putnam et al., 2005).

3.2 Identification of Central Chemoreceptors

Studying central chemoreceptors in adults is technically challenging, and more so in embryos and fetuses. Unlike the carotid body, which is fairly well accepted as the most important site for peripheral respiratory O₂ and CO₂ chemotransduction (though signal transduction mechanisms are still debated), there is disagreement about the major site(s) of brainstem chemoreceptors and their cellular mechanisms of CO₂ chemoreception. Many different putative sites of brainstem CO₂ chemoreception have been identified (fig. 1) that when focally stimulated by acid or CO₂ or acetazolamide, increase respiratory motor output (Coates et al., 1993; Corcoran et al., 2009). One school of thought is that these multiple sites, which include the retrotrapezoidal nucleus, medullary raphe, locus ceruleus, solitary tract nucleus, Pre-Bötziger complex, and caudal ventrolateral medulla, form a distributed chemoreceptor network, provide redundancy, and through interconnections, confer state dependence in the CO₂ drive to breathe. Another school of thought suggests that a particular chemoreceptive region has a pre-eminent function in chemoreception, and that the CO₂ chemosensitivity of other areas is of less importance (reviewed by Mitchell, 2004; Corcoran et al., 2009).

The retrotrapezoid nucleus (RTN), a region containing CO₂-sensitive neurons with a unique chemical phenotype, has been proposed as a candidate primary site of CO₂ central chemoreception (Guyenet and Mulkey, 2010; Mulkey et al., 2004; Guyenet et al., 2008), but this area also has extensive synaptic contact with adjacent medullary regions, and other chemoreceptive areas, so its primacy is currently debated (Mitchell, 2004; Corcoran et al., 2009). RTN chemoreceptor cells develop prenatally and are present and active at birth. RTN chemoreceptive cells have been identified as one of the targets for PHOX2B mutation that subverts the embryonic development of chemosensitivity and causes Congenital Central Hypoventilation Syndrome (see below).

Medullary raphe serotonergic neurons are another group of chemically characterized neurons that have been proposed (and debated) as a candidate primary central CO₂ chemoreceptor site. Like the RTN, this area has many synaptic inputs and outputs. The serotonergic cells here are intrinsically CO₂ sensitive even in isolation (Richerson, 2004; Richerson et al., 2005; Corcoran et al., 2009). Unlike putative RTN chemoreceptor neurons, serotonergic medullary raphe neurons in rats are relatively insensitive to acid stimulation at birth and up to postnatal day 12, then they rapidly gain CO₂/acid sensitivity to adult-levels after P12 (Wang et al., 1999). Interestingly, this developmental time course correlates with

the postnatal maturation of the adult-type HCVR. It has been proposed that RTN chemoreceptors, being fairly well developed prenatally, may be critical for the central chemoreceptor response at birth and in the perinatal period, and that the contribution of serotonin central chemoreceptors (which are more immature at birth) to the adult-type HCVR becomes more important later on (Corcoran et al., 2009).

3.3 Prenatal and neonatal recordings from presumed central chemoreceptors

Wellner-Kienitz and Shams (1998) made intracellular recordings from organotypic cultures of fetal rat medullary slices (gestational day 16 or 17). Some neurons that grew out from their cultured slices were depolarized and increased discharge during hypercapnia, and some were hyperpolarized and decreased discharge with hypercapnia. Of these neurons, a subset were intrinsically CO₂ sensitive and remained responsive even after synaptic blockade, while others became quiescent without synaptic input. The specific origin of the cultured medullary neurons was not determined, but their results are generally consistent with presence of brainstem chemosensitivity, even in embryos.

Recently, Kawai et al. (2006), using a brainstem-spinal cord preparation from neonatal rats, recorded rhythmic pre-inspiratory cells located in the caudal parafacial respiratory group (pFRG) that were depolarized and increased their intrinsic rhythmic discharge rate during respiratory and metabolic acidosis. Use of TTX suggested some of these cells were intrinsically CO₂/H⁺ sensitive, while some required synaptic input for chemosensitivity. Since parafacial pre-I cells are putative respiratory rhythm generators in embryos and neonates (along with Pre-Bötziger neurons, Thoby-Brisson et al., 2009), their intrinsic chemosensitivity may both modulate respiratory rhythm and contribute to overall central chemoreception. Onimaru et al. (2008) investigated pFRG cells in neonatal rats (0d to 2d old), focusing on phox2b-immunoreactive cells since the phox2b-expressing cells in the pFRG of the rostral medulla had been implicated in central CO₂ sensing in adult rats (Stornetta et al., 2006). The neonatal phox2b positive pFRG cells were intrinsically CO₂ sensitive as well as rhythmic. The cells did not require synaptic input for these properties, consistent with a combined role of respiratory rhythm generation and central CO₂ chemoreception. These electrophysiological studies indicate that CO₂ chemosensitivity, at least in some parts of the brainstem, develops prenatally and is functional at birth.

3.4 Congenital Central Hypoventilation Syndrome (CCHS) has provided clues about prenatal central chemoreceptor development

Known also as Ondine's Curse, CCHS is a rare genetic developmental disorder of the automatic control of breathing (Spengler et al., 2001). Symptoms of CCHS include hypoventilation, and an absence or attenuation of the normal ventilatory response to hypercapnia at birth, most prominently during sleep. Recent research into CCHS, using molecular, genetic, and neurophysiological techniques has provided insights into the development of central CO₂ chemosensitivity, which will be briefly summarized here.

CCHS is caused by mutation of the PHOX2B gene coding for the homeobox transcription factor phox2b, which is expressed in a set of central and peripheral neurons critical for autonomic development (Dauger et al., 2003). In the respiratory control system, phox2b expression is required for development of carotid bodies and their afferents, the nodose, petrosal, and geniculate visceral sensory ganglia, and the neurons in the solitary tract nucleus (Dauger et al., 2003; Stornetta et al., 2006). Phox2b is also required for prenatal development of the retrotrapezoid nucleus, a CO₂ chemoreceptive site in the rostral medulla (Guyenet et al., 2010). The RTN CO₂ sensitive cells, termed ccRTN cells ("chemically characterized", Lazarenko et al., 2009), are non-cholinergic, non-aminergic, CO₂-activated neurons that express phox2b, vesicular glutamate transporter-2 (vglut2), and substance P

NK1 receptors (Guyenet and Mulkey et al., 2010). In adult rodents the CO₂-sensitive ccRTN cells help regulate breathing rate and depth. Prenatally the ccRTN cells are part of the embryonic parafacial (e-pF) group which is an autonomous oscillator that, with the Pre-Bötziger region, helps generate embryonic respiratory rhythm. Further studies of PHOX2B gene, especially conditional expression studies, are likely to further clarify the roles of this transcription factor in the development of central CO₂ chemoreception.

Other genes and transcription factors, including MafB, Tlx1/3, Egr2, Math1, dbx1, Lbx1, atoh1, and Tshz3 have also been linked to normal development of the respiratory control system and respiratory rhythm generation in the Pre-Bötziger region (Caubit et al., 2010 ; Champagnat et al., 2010; Dubreuil et al., 2009; Thoby-Brisson et al., 2009). Investigation of the physiological genomics of respiratory control system development is still in its early stages, and holds great promise for understanding the development of central and peripheral respiratory chemoreceptors.

4. Airway Chemoreceptors

Several types of airway receptors have been described, and some, including neuroepithelial bodies (O₂-sensitive NEB in mammals and birds), slowly adapting pulmonary stretch receptors (CO₂-sensitive SAR in mammals), and intrapulmonary chemoreceptors (CO₂-sensitive IPC in birds), contribute to overall respiratory chemoreception. At present time, only the avian intrapulmonary chemoreceptors and mammalian slowly adapting pulmonary stretch receptors have been shown to produce reflex ventilatory effects in response to CO₂ (or O₂), with the avian IPC clearly the strongest airway respiratory chemoreceptor so far identified in endotherms.

4.1 Mammalian slowly adapting pulmonary stretch receptors (SAR)

SAR provide phasic feedback that helps set the depth and rate of breathing, and are the source of the Hering Breuer reflex in mammals. SAR are not only mechanosensitive, most are also CO₂ sensitive (inhibited by high CO₂, stimulated by low CO₂), which may augment their stretch sensitivity and provide for greater cyclic discharge during hypocapnia, less in hypercapnia (Coleridge et al., 1978; Farber et al., 1984; Green et al., 1986; Lai et al., 1998).

Postnatal aspects of SAR development have been studied in cat, opossum, dog, and sheep (Marlot and Duron, 1979; Farber et al., 1984; Fisher and Sant'Ambrogio, 1982; Arsenault et al., 2003). The Hering Breuer reflex is present and strong at birth and SAR sensory feedback is critical for normal breathing in the newborn; the reflex then generally becomes less important for normal breathing during maturation to adulthood (Arsenault et al., 2003). Interestingly, the postnatal maturation of SAR discharge follows an opposite pattern; SAR discharge rate is slower in neonates than in adults at any given transpulmonary pressure, and the threshold transpulmonary pressure required for activation of SAR discharge is higher in neonates (reviewed by Fisher et al., 1991).

4.2 Mammalian neuroepithelial bodies

NEB are oxygen-sensitive, vagally innervated structures containing neuroendocrine cells that are widely distributed in lung epithelium. NEB share some chemosensory attributes with carotid body glomus cells, including a depression of transmembrane K⁺ current by hypoxia, cell depolarization by hypoxia, and hypoxia-stimulated exocytosis of stored vesicles containing serotonin, CGRP, and other neuropeptides. Although most NEBs receive extensive vagal innervation, the reflex effects of hypoxic or hypercapnic stimulation of NEB are unknown. In contrast, NEB histology, histochemistry and cellular electrophysiology are quite well studied (Adriaensen et al., 2006). NEBs are among the earliest structures to develop in the embryonic lung, especially at branching points in the bronchial tree early in

lung growth, and may play a role in guiding lung development (Sorokin et al., 1997). Vagal innervation of NEB develops later in the fetus, but the role of the vagal innervation in NEB function remains obscure. Clearly, analysis of the reflex function of these complex receptors needs further study (Adriaensen et al., 2006).

4.3 Avian Airway Receptors

Stretch receptors and neuroepithelial bodies are also present in the avian respiratory system (Bouverot, 1978; Scheid and Piiper, 1986; Kubke et al., 2009), but their role in respiratory chemoreception appears to be small compared to the dominant respiratory chemoreceptors of the avian lung, the IPC (intrapulmonary chemoreceptors). Avian IPCs are specialized, CO₂ sensitive, vagal respiratory chemoreceptors that are found in the parabronchial lungs of birds. CO₂ modulation of IPC activity on a breath-by-breath basis produces nearly immediate reflex effects on breathing pattern (Fedde, 1970; Fedde et al., 1970; Banzett et al., 1977; Scheid et al., 1978; Scheid and Piiper, 1986). Comparatively, the IPC's rapid response to abruptly declining PCO₂ occurring during each inspiration in the avian lung is similar to the mammalian PSR's rapid response to mechanical stretch of the airways during inspiration (Yu, 2005). Discharge from both IPC and PSR have an inhibitory effect on inspiration which helps set the rate and depth of breathing.

Interest in avian IPC has extended over 40+ years since their discovery (Peterson and Fedde, 1968), with investigations of IPC reflex effects and discharge characteristics predominating during the early years, and CO₂ signal transduction processes investigated in the later years (Scheid and Piiper, 1986; Milsom 2002; Hempleman et al. 2004; Milsom et al. 2004; Hempleman and Pilarski, 2009). Comparatively, IPC are a relatively accessible and unambiguous model system of CO₂ chemotransduction: single-unit activity can be recorded from IPC afferent axons in the vagi, or from IPC somata in the nodose ganglia, and the local microenvironment of the IPC sensory endings can be experimentally manipulated *in situ* by controlling lung ventilation and blood flow. Because IPC have an extraordinarily high sensitivity to CO₂ compared to other respiratory chemoreceptors (Δ IPC discharge rate within each breath is typically 10–30 impulses/sec) and IPC reflex effects are pronounced, appropriately designed experiments with IPC can provide rare glimpses into the mechanisms of CO₂ signal transduction and ventilatory reflex function in intact animals.

4.4 Avian IPC Development

The development and maturation of IPC signal transduction has been studied recently in the prenatal (fig. 2A), paranatal (figs. 2B, 2C), and postnatal period (Pilarski and Hempleman, 2007). During these periods the avian embryo transitions from strictly chorioallantoic membrane (CAM) gas exchange to strictly pulmonary gas exchange, which imposes large changes in IPC microenvironment and function. Studies of this nature in mammals, even in the carotid body, are rare due to the technical difficulties accessing the embryo *in utero*, and reliably identifying individual chemosensory cells to be recorded. These difficulties are less pronounced with avian IPC

The avian hatching sequence provides an excellent but little used model for studying prenatal, paranatal, and postnatal CO₂ chemoreceptor development. While the events of avian hatching differ considerably from mammalian birth, the functional outcomes are nearly identical. Fetal mammals transition from placental to pulmonary gas exchange at birth, while avian embryos transition from CAM gas exchange to pulmonary gas exchange at hatching. This process is slower in birds, occurring over hours or days depending on the avian species, rather than seconds or minutes as in mammals. Importantly, during hatching, O₂ and CO₂ levels change in characteristic ways in the avian egg and embryo, and in many

cases can be manipulated experimentally (Visschedijk, 1968a; Visschedijk, 1968b; Tazawa et al., 1983; Mortola, 2011).

Using the mammalian carotid body as a model, Pilarski and Hempleman (2007) hypothesized that IPC function might develop prenatally and mature postnatally, perhaps triggered by the abrupt change in lung CO_2 that occurs with the conversion from CAM respiration to pulmonary respiration during hatching. During most of development, lungs are filled with amniotic fluid and intrapulmonary PCO_2 probably changes little. Relatively static and elevated PCO_2 would likely preclude spontaneous prenatal IPC discharge, but it seems likely, nevertheless, that IPC should develop enough functionality to be able to respond to the first breaths of fresh air at hatching. IPC are inhibited by elevated PCO_2 , and in the avian egg near term, the embryo is subject to a considerable CO_2 load due to its steadily increasing metabolism and the diffusion limitation imposed by the egg shell and its membranes (Mortola, 2011). Moreover, during the late prenatal and early paranatal period (fig. 2A, 2B) the high CO_2 is relatively static within the egg and embryonic lungs. This changes in the late paranatal (fig 2C) and postnatal air-breathing bird, when the lungs and IPC experience large within-breath CO_2 oscillations of 0%-5% during normal tidal ventilation (Powell et al., 1981).

The IPC response to tonic and phasic CO_2 stimuli in hatching (paranatal, fig. 2B, 2C) and newly hatched (postnatal) birds was different from the adults (fig. 3). Like the mammalian carotid body, avian IPC responses to chemostimulation changed dramatically during the postnatal period, but in contrast to the carotid bodies which have attenuated tonic and phasic discharge responses at birth, IPC have exaggerated phasic and attenuated tonic discharge responses at hatching. Adult IPC typically respond to both the rate of change in the CO_2 stimulus (phasic sensitivity) and to a constant stimulus (tonic sensitivity), fig. 3. It is unknown if the developmental trajectory of IPC is influenced by the normal changes in intrapulmonary CO_2 stimulation occurring at hatching, and it would be interesting to experimentally manipulate the prenatal, paranatal, and postnatal environments to test this.

IPC were active and CO_2 -responsive during the paranatal period (figs. 2B, 2C, 3) when the embryo first started to breathe, first from the egg's air cell (internal pipping), and then from the environment through the opened egg shell (external pipping). No IPC activity was detectable during the prenatal period (fig. 2A) before the start of pulmonary ventilation. However, it seems likely that immature IPC were present but inhibited by the partially fluid-filled lung of the prenatal, exteriorized embryo, which would interfere with the experimental delivery of low CO_2 gas in the airways. Further experiments are required to address this question.

V. Summary

Respiratory chemoreceptors, whether carotid bodies, central chemoreceptors, or airway chemoreceptors, all develop during the prenatal period and are present at birth (or hatching) with sufficient functionality to support the conversion from placental or chorioallantoic gas exchange to pulmonary gas exchange. Failures of prenatal respiratory chemoreceptor development are associated with serious or fatal deficits in respiratory control, including CCHS (Ondine's curse) and possibly SIDS. Even in normal newborns, a period of postnatal maturation is required for carotid bodies, central chemoreceptors, and IPC before the final adult-type chemoreceptor response to O_2 , CO_2 and/or pH is seen. Evidence in carotid bodies indicates this maturation or "resetting" depends on the characteristic changes in inspired PO_2 and/or PCO_2 that occur with the state change in respiration at birth, and this state change is likely to affect other chemoreceptors as well (an area for further study). Recent experiments show that the development of respiratory chemoreceptors is programmed by a

host of transcription factors whose genes must be expressed with temporal and spatial precision to produce a normal respiratory control system. This is an exciting research area in respiratory chemoreceptor development, and continued investigation of the developmental physiological genomics of the respiratory control system holds great promise for the future.

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Fig. 1.

Respiratory-related regions of the mammalian brainstem. VIIIn, cranial nerve 7 (facial); Vn, cranial nerve 5 (trigeminal); Mo5, trigeminal motor nucleus; KF, Kölliker-Fuse nucleus; PBr, parabrachial nucleus; A5/VLP, area 5 noradrenergic group; CCA, caudal cerebellar artery, r2–3, rhombomere 2–3, r4–5, rhombomere 4–5; RTN/pFRG, retrotrapezoid nucleus/parafacial respiratory group; 7, facial nucleus; NTS, nucleus of the solitary tract, BötC, Bötzinger complex; preBötC, preBötzinger complex; NA, nucleus ambiguus; LRt, lateral reticular formation; VRG, ventral respiratory group; XIIIn, cranial nerve 12 (hypoglossal); XII, hypoglossal motor nucleus; raphe, medullary raphe, BA, basilar artery, C1, 1st cervical root, IX, cranial nerve 9 (glossopharyngeal); X, cranial nerve 10 (vagus). Modified from Rekling and Feldman (1998)

Fig. 2.

Anas platyrhynchos hatching sequence. (A) Prenatal period: prior to internal pipping, gas exchange is provided exclusively by the chorioallantoic membrane (CAM). (B) Early paranatal period (internal pipping). Gas exchange is shared by the CAM and the lungs. Embryo breathes gas from the air cell at the blunt end of the egg, which is both hypoxic and hypercapnic, and extracellular pH falls during this period. (C) Late paranatal period (external pipping). Gas exchange is shared by CAM (decreasing importance) and lungs (increasing importance). The embryo has pipped through the shell and breathes atmospheric air, providing relief from hypoxia and hypercapnia. 1. air cell. 2. inner shell membrane 3. chorioallantoic membrane. See text for more detail and also Mortola, 2011. Pencil illustrations were drawn by Northern Arizona University graduate student Maureen L. Maloney. Reproduced with permission from Elsevier Limited (original ref. Pilarski and Hempleman, 2007).

Fig. 3.

Summary data for IPC response to CO₂ during development. Cycle-triggered stimulus histograms of IPC action potential discharge as inspired CO₂ was stepped between 0% and 7% (11 s stimulus cycle, 0.250 s bin width, two cycles shown, n is number of IPC single units studied). Points show **mean $\pm$ sem**. Note the blunted tonic and enhanced phasic IPC CO₂ sensitivity during the paranatal period (internal and external pipping), and early postnatal period, and the maturation of this characteristic after hatching. Reproduced with permission from Elsevier Limited (original ref. Pilarski and Hempleman, 2007).