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Neonatal maturation of the hypercapnic ventilatory response and central neural CO₂ chemosensitivity

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Abstract

The ventilatory response to CO₂ changes as a function of neonatal development. In rats, a ventilatory response to CO₂ is present in the first 5 days of life, but this ventilatory response to CO₂ wanes and reaches its lowest point around postnatal day 8. Subsequently, the ventilatory response to CO₂ rises towards adult levels. Similar patterns in the ventilatory response to CO₂ are seen in some other species, although some animals do not exhibit all of these phases. Different developmental patterns of the ventilatory response to CO₂ may be related to the state of development of the animal at birth. The triphasic pattern of responsiveness (early decline, a nadir, and subsequent achievement of adult levels of responsiveness) may arise from the development of several processes, including central neural mechanisms, gas exchange, the neuromuscular junction, respiratory muscles and respiratory mechanics. We only discuss central neural mechanisms here, including altered CO₂ sensitivity of neurons among the various sites of central CO₂ chemosensitivity, changes in astrocytic function during development, the maturation of electrical and chemical synaptic mechanisms (both inhibitory and excitatory mechanisms) or changes in the integration of chemosensory information originating from peripheral and multiple central CO₂ chemosensory sites. Among these central processes, the maturation of synaptic mechanisms seems most important and the relative maturation of synaptic processes may also determine how plastic the response to CO₂ is at any particular age.

Keywords

Mammals; Ventilation; Tidal volume

1. Introduction

Mammals are born immature, but the extent of immaturity varies among different species. Many mammalian newborns are altricial and quite helpless. In addition to the obvious physical and behavioral immaturity of newborn mammals, many homeostatic processes are poorly developed at birth. This has often led to the assumption that the development of homeostatic processes is linear, progressing from a poorly developed neonatal form (i.e. imperfect adult

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state) to a fully adult state. However, many homeostatic processes have uniquely neonatal forms. Among these processes, thermoregulation and metabolic activity often have features that are unique to newborns. Respiratory responses to hypercapnia and hypoxia are part of a general scheme aimed at maintaining metabolic homeostasis, and we should not be surprised that the ventilatory responses to hypoxia and hypercapnia differ in newborn mammals compared to adults. We have been particularly interested in the ventilatory response to CO₂. In this review, we will first describe the development of the acute and chronic ventilatory responses to CO₂ and then speculate on the reasons for this developmental progression.

2. Development of central chemosensitivity

2.1. The acute response to hypercapnia

The ventilatory response to CO₂ in newborns is fundamentally different from the newborn response to hypoxia. Hypercapnia stimulates ventilation in general, but it seems to elicit little or no metabolic response that might modify the demand for ventilation. In contrast, hypoxia may stimulate ventilation initially, but a part of the coordinated response to hypoxia in newborns includes significant reductions in body temperature and metabolic rate (Mortola and Lanthier, 1996; Saiki and Mortola, 1996). Although the ventilatory response to CO₂ may lack a metabolic component and is generally stable during the short duration exposure periods that have been studied, the ventilatory response to CO₂ is not stable over the period of development. Early reports in humans indicated that the ventilatory response to CO₂ was reduced in premature infants (the infants had a limited respiratory frequency (FR) response to increased CO₂, but a more substantial tidal volume (VT) response). The ventilatory response to CO₂ increased as the infants matured (Krauss et al., 1975; Rigatto et al., 1975; Frantz et al., 1976). In term infants, the ventilatory response to CO₂ may increase slightly over the first 8 weeks of life, but the newborn response (P2; postnatal day 2) is close to the mature value (Søvik and Lossius, 2004). Subsequent work in dogs confirmed that the ventilatory response to CO₂, though there was an increase in FR and VT, was still less than the adult response (Nattie and Edwards, 1981). The situation is a bit confusing in piglets. Anesthetized piglets (P2–P11) appear to have a more adult response pattern in which the ventilatory response to CO₂ is just as vigorous on P2 as it is in adults (Wolsink et al., 1992). However, the ventilatory response to CO₂ in intact piglets may reach its lowest point around P15 (Nattie, unpublished observations), and studies that are curtailed before 2 weeks of age may miss the developmental progression of ventilatory responses to CO₂ in piglets.

The ventilatory response to hypercapnia has been studied most thoroughly as a function of age in rats. Early reports indicated that the hypercapnic response was very modest in the first 2 postnatal weeks (Bamford et al., 1996). There was little effect of CO₂ on frequency early in development (VT did increase), but the frequency response increased by P18, and the development of an increased ventilatory response to CO₂ over the first weeks of life tracked the emergence of a frequency response to CO₂ (Bamford et al., 1996; Abu-Shaweesh et al., 1999). Stunden et al. (2001) described changes in ventilation during hypercapnia in neonatal rats ranging in age from P1 to P21 and in animals greater than 100 days of age. Initially, the response to CO₂ was quite vigorous (P1–P5), but subsequently, it diminished and reached its lowest point at P8 (Fig. 1). Thereafter, the response rose until P21, when it seemed to arrive at the mature level (Fig. 1). The ventilatory response to CO₂ was dominated by increases in VT; FR increased only modestly in the early newborn period (Saiki and Mortola, 1996; Stunden et al., 2001). Other studies have found a similar pattern in newborn rats although the details of the VT versus FR responses varied among studies. Serra et al. (2001) reported that there was a modest ventilatory response in the immediate postnatal period (P1–P3), consisting of both increased VT and FR. Approximately, 1 week after birth (P6–P7) virtually, no response to CO₂ existed. Subsequently, the response to CO₂ returned and approached adult levels after 3–4 weeks of age. Wickström et al. (2002) also found that the ventilatory response to CO₂ was

at a minimum at P7 in rats. In this study, the ventilatory response to CO₂ in younger animals arose from an increase in FR (rather than VT as previous studies reported), but the response to CO₂ still fell between P7 and P10. There was a slow increase in the VT response to CO₂, so that VT became more important in older rats.

Responses in mice differ slightly from rats in that no clear nadir is seen at 1 week of age, but the response is similar in that the postnatal response to CO₂ is less than the response at 2–3 weeks of age. Furthermore, the ventilatory response to CO₂ may not be sustained in mice. During a 5 min exposure to a fractional inspired CO₂ (FICO₂) of 5%, VT was elevated and remained constant, but FR declined over 5 min in the P6 age group during the hypercapnic exposure. Mice that lack one of the synthetic enzymes for γ -amino-butyric acid (the 65 kD form of glutamic acid decarboxylase; GAD65) had a sustained frequency response to hypercapnia at P6. Thus, the presumed lack of inhibitory neurotransmitter release seemed to sustain the FR response to CO₂ (Bissonnette and Knopp, 2004).

In summary, there seem to be at least two and possibly three phases in development of the ventilatory response to CO₂ (Fig. 1). In rat pups, the ventilatory response actually declines in the first postnatal week (phase I). In other species, this decline is not apparent, but in all species, the CO₂ response is low in newborns between the first and second week of age (phase II) and then increases toward the adult level during subsequent development (phase III). The relative importance of FR or VT in the response to CO₂ is variable among studies, but in rats, most investigators report that a VT response is present at birth, but a FR response to CO₂, if present, wanes in the first few days of life and returns only as the adult pattern of responsiveness emerges. Part of the maturation of the ventilatory response seems to be associated with the emergence of a FR response to CO₂.

2.2. Developmental patterns in non-mammals

Among other phyla, the response to CO₂ may vary significantly over the course of development. For example, the CO₂ response in larval insects often involves control of spiracular opening, but gas exchange, even in the tracheolar system of relatively large pupa depends on diffusion only (Kanwisher, 1966; Levy and Schneiderman, 1966). Metamorphosis into the adult form brings about a marked change in the respiratory pattern among larger insects. Larger adult insects, such as cockroaches, actively expire, and the elastic characteristics of the exoskeleton recoil and draw air into the tracheolar system during the passive inspiratory phase of the respiratory cycle. Hypercapnia stimulates active expiratory efforts and coordinates the opening and closing of individual spiracles to create unidirectional ventilation of the tracheolar system in the adult cockroach (Schreuder and De Wilde, 1952; Miller, 1973). Thus, in large insects that go through complete metamorphosis, the entire pattern of hypercapnic responses may change (spiracular control of diffusion to active pumping plus spiracular control), and in those insects that go through incomplete metamorphosis, the role of active ventilation grows as the size of each successive instar increases.

Metamorphosis also transforms the ventilatory response to CO₂ in frogs. Frogs manifest two patterns of breathing, which can be discerned even in the isolated tadpole brainstem (Torgerson et al., 1998). One pattern of cranial nerve activity reflects the gill rhythm and the other reflects the rhythm of lung inflation. The early stages of amphibian metamorphosis are purely aquatic, and gill and skin ventilation dominate gas exchange. Aquatic animals usually have low PCO₂ levels (~3–5 mmHg), and hypercapnia does not modify the pattern of fictive gill ventilation in the isolated tadpole brainstem preparation (Taylor et al., 2003a,b). As metamorphosis progresses, the blood PCO₂ rises in tadpoles (Just et al., 1973), and the frequency of bursts of lung-related cranial nerve activity in the isolated tadpole brainstem also increases and lung bursts begin to occur in clusters. Further, hypercapnia stimulates lung burst activity but not gill bursting activity (Taylor et al., 2003a,b). Lung bursts are infrequent in the

early stages of metamorphosis, but they seem to be actively inhibited, since a combination of bicuculline and strychnine (antagonists of GABA and glycine) increased the frequency of lung bursts even in the early stages of metamorphosis (Straus et al., 2000). The effect of inhibition of GABAergic mechanisms is similar in tadpoles and in neonatal mice (Bissonnette and Knopp, 2004) in that the frequency response is unleashed. Thus, the processes responsible for the early developmental pattern of the hypercapnic ventilatory response in mammals may be similar in some other classes.

2.3. Neonatal development of other autonomic control systems

Like the ventilatory response to hypercapnia, a variety of other systems, such as the gustatory system, autonomic regulation of the cardiovascular system and baroreceptor responses, exhibit neonatal patterns of change during early postnatal development in the rat. The development of the gustatory system has been studied by exposing rats of different ages to various taste stimuli ("sweet", "bitter and sour" and "salt") and noting their orofacial responses (Stewart and Hill, 1993). The rats could detect the various stimuli by P5. They could discriminate between water and sucrose ("sweet") within the first postnatal week, but did not develop the mature preference for sucrose until P15 (Stewart and Hill, 1993). In contrast, it was not until P9 before rats could discriminate between water and quinine ("bitter") (Stewart and Hill, 1993). Interestingly, the ability of the rats to taste salt had a triphasic developmental pattern. At P3, rats rejected the salt stimulus (Stewart and Hill, 1993). However, rats aged P10 seemed to prefer NaCl-containing solution and this preference seemed to remain at least until P25. By P48, rats once again rejected salt stimuli and appeared to reach an adult-like distaste for NaCl-containing solutions (Stewart and Hill, 1993). Thus, the taste response shows a complex developmental pattern and is not fully mature at birth.

Like the gustatory system, the autonomic responses that control the cardiovascular system appear to develop postnatally. For instance, the heart rate of young rats (P3–P5) does not respond to handling stress, while older rats (P6–P16) show a decreased heart rate in response to handling stress (Hofer and Reiser, 1969). The heart rate response reaches an adult state only after P20, when handling elicits an increase in heart rate (Hofer and Reiser, 1969). Once again, a complex developmental pattern is displayed in autonomic regulation of the cardiovascular system that is not fully developed at birth.

A triphasic pattern of development, as seen during maturation of the hypercapnic ventilatory response in rats, was also seen in studies of the baroreceptor reflex response to phenylephrine infusion. A baroreflex response was seen in young rats (P5–P6; heart rate slowed significantly), but the response was attenuated in older rats (P10–P20). After P20, the baroreflex was once again evident, and the heart rate decreased to levels similar to P5–P6 rats after phenylephrine administration (Kasparov and Paton, 1997). During the period of reduced baroreflex responses, arterial blood pressure increased from relatively low neonatal values to higher, adult levels (Kasparov and Paton, 1997).

In summary, all of these systems have something like a triphasic developmental profile: responsive at birth, then a period of reduced responsiveness, and finally an adult pattern of sensitivity. Thus, a complex, non-linear neonatal pattern of development in the hypercapnic response of ventilation is not unusual compared to the non-linear development of other autonomic functions.

2.4. Modification of the development of central chemosensitivity

There has been a long-standing debate over which facets of behavior are inborn and immutable and which facets of behavior are plastic and modified by experience. This 'nature versus nurture' debate has been studied in the respiratory response to CO₂. Investigations into this

question were prompted by the observation that fossorial animals often have blunted ventilatory responses to CO₂ (Boggs et al., 1984). The burrows of fossorial animals have elevated CO₂ levels, and investigators wondered whether the reduced responsiveness to CO₂ resulted from acid–base adjustments following persistent inhalation of elevated CO₂ (nurture) or evolutionary selection and an inborn insensitivity to level.(nature). The initial efforts to modify the CO₂ responsiveness of newborn animals involved maternal exposure to hypercapnic conditions during pregnancy and then exposure of the newborn during the pre-weaning period (3 weeks). The ventilatory response of the newborn to CO₂ was tested at least 6 weeks after room air conditions were restored to allow any acid–base adjustments (CO₂ retention) to dissipate. The initial studies revealed no evidence of CO₂ retention after exposure to CO₂ in utero or after exposure for 3 weeks after birth in either mice or rats. Adult rats exposed to increased CO₂ for 3 weeks also demonstrated no change in arterial PCO₂ or the ventilatory response to CO₂ (Birchard et al., 1984). These authors concluded that reduced CO₂ responsiveness was an inborn trait (nature triumphing over nurture). Subsequent studies indicate that nurture may still play a role in some circumstances. Chronic hypercapnic exposure of zebra finches when still embryonic or in the nest (exposure of the eggs or newborn hatchlings) seemed to reduce the ventilatory response to CO₂ even when CO₂ sensitivity was assessed after months of normocapnic exposure (Williams and Kilgore, 1992). On the other hand, the ventilatory response to CO₂ was diminished only in the females of one strain of quail after embryonic exposure to 2% CO₂ (Bavis and Kilgore, 2001).

An alternative approach has been to expose newborn animals to briefer periods of hypercapnia and then examine the response to CO₂ later in life to determine if there is a window of vulnerability when the CO₂ response is mutable within the time of development. From these studies, it seems that the pattern of development of the ventilatory response to hypercapnia is not entirely genetically determined, but can be modified. The clearest example of this is that exposure of rats to chronic hypercapnia results in changes in the ventilatory response to acute inspired hypercapnia. For instance, neonatal rats exposed for 7 days (P0–P7) to an environment with 7% CO₂ had a reduced ventilatory response to acute hypercapnic exposure when tested 2 days after returning to room air, suggesting that chronic hypercapnia suppressed central chemosensitivity (Rezzonico and Mortola, 1989). However, it could be argued that acid–base adaptations to hypercapnia may not resolve in 2 days, as the authors noted. More recently, the effects of different chronic hypercapnic protocols on the development of ventilatory responses to acute hypercapnia have been studied. Using a protocol similar to that of Rezzonico and Mortola (1989), exposure of neonatal Sprague–Dawley rats (P0–P1) for 7 days to 7.5% CO₂ (chronic hypercapnic protocol 2, CHC-2), resulted in a complete suppression of the acute ventilatory response to inspired hypercapnia in rats aged P7–P20 even though the rats were kept in room air conditions after P8 (Nichols et al., 2005) (Fig. 2). In a different protocol, the chronic hypercapnic (7.5% CO₂) exposure was started approximately 5 days before birth (exposure of pregnant mother before birth) and continued until the pup was used in an experiment (chronic hypercapnic protocol 1, CHC-1). In these persistently hypercapnic animals, the normal triphasic pattern of ventilatory response to acute inspired hypercapnia was seen, but the nadir in the responsiveness to inhaled CO₂ occurred at P9–P12 rather than P6–P8 (Nichols et al., 2005) (Fig. 2). These data suggest that chronic hypercapnia has a different effect on the ventilatory response to hypercapnia when initiated before birth compared to postnatal hypercapnia, but even the effect of postnatal hypercapnia depends a great deal on the age at which the animal is exposed to hypercapnia. It is likely that the early development of the hypercapnic ventilatory response can also be affected by other environmental factors, such as oxidative stress (Dean et al., 2003; Mulkey et al., 2003), hypoxia (McGregor et al., 1998; Simard et al., 2003), and low birth weight or pre-maturity (Davey et al., 1996).

In mammals at least, fetal exposure to elevated CO₂ and exposure of adults to CO₂ seem to have no lasting effect on the ventilatory response to CO₂ (Birchard et al., 1984). Synaptogenesis

does not begin until relatively late in embryonic development in rats (Zhang and Ashwell, 2001), and the major change in synapses later in life consists of a process of synaptic elimination and pruning as only favored synaptic pathways are selected. It is tempting, therefore, to speculate that ventilatory plasticity is more likely to persist from the neonatal period because it is only during this time that synaptogenesis is occurring and only at this time that environmentally induced modifications of gene expression can have a lasting effect on behavioral responses to CO₂. Ventilatory plasticity in the hypercapnic response is, therefore, most likely to occur when synapses are present or being formed, but before the pattern of synaptic connectivity is pruned and fixed.

3. Neural mechanisms of development of ventilatory responses to CO₂

Given these patterns of development of the ventilatory response to CO₂, what are the possible mechanisms whereby these changes might come about? There are several developmental processes that could be involved, including changes in central chemosensitivity, respiratory muscle or lung mechanics, the neuromuscular junction or gas exchange. We will restrict our consideration only to central changes. There seem to be four possible neural mechanisms to us. First, there may be developmental changes in the function of CO₂ chemosensory neurons. Second, astrocytes appear to modify chemosensory function, and astrocytic processes go through a maturational process as well. Third, both electrical and chemical synaptic mechanisms may mature, and the relative role of excitatory and inhibitory synaptic mechanisms may change over development. Finally, the relative importance of different chemosensory sites, both peripheral and central, may change during development. Each of these possibilities will be discussed in turn. We want to emphasize that the maturation of CO₂ sensitivity is unlikely to have a single explanation, and the possible mechanisms that we have identified are not mutually exclusive nor do they exclude other mechanisms, such as mechanical changes, not discussed here.

3.1. Developmental changes in neurons from various chemosensitive brainstem regions

As suggested above, the developmental changes in the ventilatory response to hypercapnia could be due, in part, to either developmental changes of the responsiveness of individual chemosensitive neurons to hypercapnia or to a developmental change in the number of chemosensitive neurons. While no neurons in mammals have been unequivocally identified as chemosensitive neurons involved in ventilatory control, based on the ventilatory response to focal acidification CO₂-sensitive neurons have been located in a number of brainstem regions, including the ventrolateral medulla, the NTS, the medullary raphé, the retrotrapezoid nucleus and the locus coeruleus (Feldman et al., 2003). In each of these regions, neurons have been found whose firing rate is altered substantially upon exposure to hypercapnia (Dean et al., 1989, 1990; Richerson, 1995; Pineda and Aghajanian, 1997; Oyamada et al., 1998; Wellner-Kienitz and Shams, 1998; Filosa et al., 2002; Mulkey et al., 2004; Putnam et al., 2005).

The development of chemosensitivity has only been studied electrophysiologically in neurons from three brainstem regions: the medullary raphé (Wang and Richerson, 1999), the locus coeruleus (Stunden et al., 2001), and the solitary tract (Conrad et al., 2005). Wang and Richerson (1999) studied the appearance of chemosensitive neurons in slices from the medullary raphé of neonatal rats. The percentage of neurons stimulated by hypercapnia was significantly greater in slices from rats older than P12 (18%) compared to rats younger than P12 (3%), while the percentage of neurons inhibited by hypercapnia was about the same in slices from rats of all ages (16%). These findings were paralleled in medullary raphé neurons in tissue culture. Fewer raphé neurons in culture for less than 12 days were stimulated by hypercapnia than in neurons in culture for greater than 12 days (4% versus 30%) (Wang and Richerson, 1999). Further, the relative increase in the firing rate per unit estimated pH change (the chemosensitivity index expressed as a percent) of both stimulated and inhibited raphé

neurons (Wang et al., 1998), increased in cultured raphé neurons with days in culture. For instance, the chemosensitivity index of raphé neurons in culture for less than 12 days increased from <200 to ~300% in neurons in culture for greater than 18 days, while it decreased in raphé neurons that were inhibited by elevated CO₂ from ~60 to ~20% over the same time period (Wang and Richerson, 1999). Based on these findings, both the number of chemosensitive neurons and the magnitude of their response to hypercapnia increase during early development in the medullary raphé. These findings are at odds with studies described below in which Fos labeling was used to estimate the number of CO₂-sensitive neurons (Belegu et al., 1999).

A different pattern has been observed in neurons from the locus coeruleus. Individual LC neurons in brainstem slices from Sprague–Dawley rats of different ages were studied with perforated patch electrodes (Stunden et al., 2001). Virtually, all LC neurons respond to hypercapnia with an increased firing rate (Oyamada et al., 1998; Filosa et al., 2002), and this was the case in LC neurons from neonatal rats aged P1–P21 as well as in adults (Stunden et al., 2001). Further, the degree of increase in firing rate during the hypercapnic exposure (15% CO₂) was also the same in neurons from rats aged P1–P21 (Stunden et al., 2001) and in adults (Elam et al., 1981; Pineda and Aghajanian, 1997). These findings indicate that, unlike neurons from the medullary raphé, chemosensitivity is fully developed at birth in LC neurons and shows no evident changes during development.

Recently, electrophysiological studies have been performed in neurons within the NTS. Hypercapnia (15% CO₂) activated about 40–50% of NTS neurons, regardless of the age of the rat from which a slice was taken (from P1 through adults) and regardless of the method of measurement, either perforated or whole cell patch pipettes (Conrad et al., 2005). Further, the chemosensitivity index does not differ in NTS neurons from rats of different ages; it is between 130 and 170% in all age groups. The small percentage (5–10%) of neurons inhibited by hypercapnia also is stable throughout development (Conrad et al., 2005). Thus, like LC neurons, chemosensitive NTS neurons are fully developed at birth and do not appear to change their properties with development.

Belegu et al. (1999) and Wickström et al. (2002) used a different, non-electrophysiological approach to study the development of chemosensitivity in different brainstem regions by using Fos labeling to identify cells activated by hypercapnia. This method is not quantitative; it identifies the number of cells that are activated by CO₂, but indicates nothing about the relative sensitivity to CO₂ among the Fos-positive cells. Furthermore, Fos positivity may reflect the activation of intrinsically CO₂-sensitive neurons, but it may also reflect synaptically driven activity from other sites, such as the carotid body. Belegu et al. (1999) studied rat pups at P5, P15 and P40 after exposure to 10% inhaled CO₂ for 1 h. They found increased numbers of Fos (+) cells after hypercapnic stimulation in the NTS, along the ventral surface of the brainstem, the RTN and the midline raphé. The number of stimulated Fos(+) neurons was stable across ages P5–P40 except in the raphé nuclei where the number of CO₂-stimulated neurons declined as animals matured—the opposite of the effect described by Wang and Richerson (1999).

Wickström et al. (2002) used a similar approach in which c-Fos RNA labeling was studied after exposing rat pups aged P1–P10 to 5% CO₂ for 1 h. The number of CO₂-sensitive cells was stable from P1 to P10 in the nucleus tractus solitarius, parvocellular lateral reticular nucleus, raphé pallidus, and rostral ventrolateral medulla. The number of CO₂-sensitive cells did increase in the lateral reticular nucleus as animals grew older. The c-Fos labeling was less in the midline raphé than Belegu et al. (1999) described, but in neither study were CO₂-sensitive neurons identified in the locus coeruleus (Wickström et al., 2002).

In summary, the number of CO₂-sensitive neurons and the CO₂ sensitivity of each neuron seems to be stable during development in most identified CO₂-sensitive regions. The

exceptions are the parvocellular lateral reticular nucleus, where the number of CO₂-sensitive cells increases, and the raphé nuclei, where the number of CO₂-sensitive neurons has not changed consistently among studies, but where the CO₂ sensitivity of individual neurons may increase during development. On the whole, it is difficult to attribute the complex pattern of ventilatory responsiveness to CO₂ to a developmental change in either the number or sensitivity of chemosensory cells. Neuronal responsiveness to CO₂ seems to be present at birth and generally stable during the period of development (Fig. 3), when the whole animal ventilatory response to CO₂ is changing dramatically.

3.2. Developmental changes in the role of astrocytes in chemosensitivity

Astrocytic proliferation occurs between days P8–P12 in rodents (Fig. 3). As astrocytes proliferate and mature, their contribution to the regulation of the extracellular environment becomes apparent. Astrocytes participate in the processing of neurotransmitters, such as glutamate and ATP; they synthesize and release lactate as part of an astrocytic–neuronal lactate shuttle; and astrocytes maintain extracellular potassium and pH balance. Any one of these processes might alter the function of central chemosensory neurons. For example, extracellular pH declines in the spinal cord as astrocytes proliferate (Jendelová and Syková, 1991), and prevention of astrocytic proliferation prevents the decline in pH (Syková et al., 1992). The proliferation of astrocytes is also temporally associated with a loss of pH regulatory function in neurons within the brainstem (Nottingham et al., 2001). A variety of astrocytic transport processes might have a net acidifying effect on extracellular pH (pH_e) and thereby change the level of chemosensory stimulation for any level of inhaled CO₂. Our own studies are, however, equivocal with respect to this hypothesis. In electrophysiological studies in younger animals, we found little evidence of astrocytic depolarization or pH_i regulation during hypercapnia in the RTN (Ritucci et al., 2005); some evidence of regulation of pH_i must be present if astrocytes modify pH_e. However, we have seen evidence of regulation of pH_i during hypercapnia in a subset of astrocytes in older mice (Erllichman, unpublished observations). To the extent that a subset of astrocytes regulate pH_i during hypercapnia, they will amplify the acidification of pH_e and reduce the pH in the environment of chemosensory neurons. Moreover, disrupting astrocytic function with astrocyte-specific metabolic poisons acidifies extracellular pH and stimulates ventilation (Erllichman et al., 1998; Holleran et al., 2001). Thus, the proliferation and maturation of astrocytes may change the chemosensory environment and amplify any hypercapnic stimulus. These amplifying effects will develop after P8 and may contribute to the increased ventilatory response to CO₂ after that age (phase III).

3.3. Development of brainstem neurons and synaptogenesis

Neurons undergo marked electrophysiological and morphological changes during early development. Using the nucleus tractus solitarius (NTS) of rats as an example, dramatic morphological changes are seen in somal size, dendritic length, and spine density during the first 3 weeks of life (P0–P21) (Vincent and Tell, 1997). Somal size of NTS neurons is roughly constant up to about P12, but grows rapidly after that, nearly doubling before reaching a stable adult size. While somal size increases after P12, dendritic spines (small extensions on the dendrites) reach a maximum density at P12 and decrease in number until adulthood (Vincent and Tell, 1997). The dendrites from which spines project increase in length after P15, mostly due to increases in the length of secondary and tertiary dendrites (Vincent and Tell, 1997). The particular patterns of activity present in the central nervous system seem to prune the dendritic arbor and decrease the number of synapses, so that certain synapses are strengthened and others are removed. Glutamate, acting through NMDA receptors, seems to be particularly important in the process of stabilizing favored synaptic pathways while eliminating others (Vincent et al., 2004). The process of synaptic development is active in the early postnatal period, but extends well beyond P21 (Lachamp et al., 2002). For example, the density of synaptophysin, a synapse-specific protein, appears to increase approximately three-fold in the NTS between

P0 and P9, and there is a further two-fold increase in synaptophysin density between P9 and P30 (Rao et al., 1999). Similar changes in synaptophysin density were found in the nucleus ambiguus and ventrolateral medulla. The number of pre-synaptic boutons also increases between P30 and adulthood (Miller et al., 1982). Thus, rat NTS neuronal morphology changes substantially during the first few weeks of life.

Morphology is not the only NTS neuronal property to change during early development. These neurons undergo considerable electrophysiological changes during the postnatal period as well. Vincent and Tell (1997) found that action potential waveforms change during the first postnatal month. In P0 neurons, the action potentials were smaller in amplitude and longer in duration than in more mature neurons. Action potentials appeared to reach a maximum peak in amplitude at approximately P11–P14, and then decreased to a smaller adult amplitude (Vincent and Tell, 1997). These differences in action potential amplitude suggest that Na⁺ channel density increases during development. Vincent and Tell (1997) also found that both the time constant and input resistance of NTS neuronal membranes decreased in rats older than P21; the input resistance decreased by nearly half from P0 to P21. In addition, the rheobase (the minimum current needed to induce an action potential) was nearly twice as high in NTS neurons from adult rats as compared to rats aged P0–P21 (Vincent and Tell, 1997). These findings suggest that it is easier for current to pass through the neuronal membrane as development progresses. This may result from increasing ion channel density as a function of age in NTS neurons. An increase in ion channel density could either increase or decrease chemosensitivity, depending on which ion channels are increased and on their intrinsic pH sensitivity. Nevertheless, the observed change in rheobase implies that the intrinsic CO₂ sensitivity of chemosensory neurons might be expected to decrease as animals develop. This, however, does not seem to be the case (see Section 3.1).

3.3.1. The maturation of synaptic mechanisms: electrical synapses—Gap junctions are ubiquitous in CO₂-sensitive regions of the brainstem (Solomon et al., 2001; Solomon, 2003). Moreover, early in development, neurons may form large aggregates of cells all interconnected by gap junctions (Connors et al., 1983; Yuste et al., 1995). As the animal ages and the networks within the brain mature, the number of interconnected cells declines dramatically (Connors et al., 1983; Christie et al., 1989; Mazza et al., 1992). These large collections of neurons connected by electrical synapses seem to be an important form of intercellular communication in the formation of neural networks (Yuste et al., 1995). Functional gap junctions are demonstrable in some CO₂ chemosensory regions (Dean et al., 1997; Huang et al., 1997), and these gap junctions may enhance neuronal CO₂ sensitivity. Having said that, if the capacitance of coupled cells is large, gap junctions could actually inhibit neuronal activity (Kepler et al., 1990). Furthermore, gap junctions play a particularly prominent role coordinating GABAergic inhibitory networks (Long et al., 2004). Thus, even though gap junctions coordinate or amplify the output of a set of neurons, the net effect may still be inhibitory. Nonetheless, disruption of gap junctions in animals aged 7–9 weeks was associated with a blunted ventilatory response to CO₂ (Hewitt et al., 2004; Parisian et al., 2004). There are no studies analyzing the role of gap junctions in intact rat pups, but it seems likely that gap junctions between CO₂-sensitive cells augment the respiratory response to CO₂ even in these very young animals, since gap junctions are clearly and preferentially present between CO₂-sensitive cells in the NTS at a young age (Dean et al., 1997; Huang et al., 1997).

The number of neurons connected by gap junctions diminishes during development (Connors et al., 1983; Christie et al., 1989; Mazza et al., 1992), and it seems likely that the amplifying effect of gap junctions declines as animals mature. There is evidence of a developmental decline in the contribution of gap junctions to hypercapnic ventilatory responses to CO₂ in the RTN in animals older than 10 weeks of age (Hewitt et al., 2004; Parisian et al., 2004). We suspect that part of the early decline in CO₂ sensitivity may reflect the initial loss of gap junctional

connectivity among CO₂-sensitive neurons although this has not been studied explicitly in very young animals. The declining role of gap junctions in CO₂ responses extends well past the nadir of the ventilatory response to CO₂ that has been observed in young rats (Stunden et al., 2001). Therefore, the loss of gap junctions could contribute to the initial decline in ventilatory responsiveness to CO₂, but other excitatory influences develop that seem to replace the excitatory function of gap junctions in older animals.

3.3.2. Maturation of ‘inhibitory’ chemical synapses—In the early postnatal period of rats, the ventilatory response to CO₂ declines and then recovers over the first 3–4 weeks of life. The timing of this decline and recovery corresponds to major events in synaptic development. In many neural networks, GABA is the initial neurotransmitter, and it is excitatory rather than inhibitory (Ben-Ari, 2002) (Fig. 3). The intracellular chloride concentration is high in the immediate postnatal period in rats, and intracellular chloride does not decline to adult intracellular levels in cortical slices until approximately P14 (Owens et al., 1996). The developmental decline in intracellular chloride is caused by an increase in the expression of the K⁺–Cl[–] cotransporter, which extrudes Cl[–] into the extracellular space (Rivera et al., 1999). Even as the intracellular Cl[–] concentration is declining, the resting membrane potential and action potential threshold seem to be relatively stable. Therefore, the changes in Cl[–] concentration significantly alter the response of neurons to GABA, and as a consequence, activation of ionotropic GABA receptors is excitatory in young animals (chloride moves down its electrochemical gradient, leaves the cell and depolarizes the cell in the process).

An early excitatory effect of GABA has been shown for hippocampal neurons (Gaiarsa et al., 1995), neocortical neurons (Luhmann and Prince, 1991), and phrenic motor neurons (Su and Chai, 1998). It is tempting to speculate that at least part of the decline in ventilatory responses to CO₂ seen in rats in the first week of life reflect the loss of excitatory GABAergic inputs in the first 2 weeks of postnatal life. However, there are conflicting reports about whether or not GABA is excitatory early in development. Some investigators have interpreted the poor frequency response to inhaled CO₂ in the early postnatal period as evidence of inhibitory GABAergic effects, since bicuculline, a GABA_A antagonist, enhanced the frequency response to CO₂ even in P5 rats (Abu-Shaweesh et al., 1999). Furthermore, Kim et al. (1997) showed that GABA occasionally depolarizes rostral NTS neurons from young rats, while Grabauskas and Bradley (2001) found that rostral NTS neurons from young rats hyperpolarized in the same manner as adult neurons in response to GABA. In ventral respiratory group neurons from rats, hyperpolarizing Cl[–] currents were seen at P0, and therefore, these neurons have an inhibitory GABA response (Brockhaus and Ballanyi, 1998). In contrast, Ritter and Zhang (2000) found GABAergic depolarization in immature mouse pre-Bötzinger complex neurons. This GABA-induced depolarization continued until P3, when the reversal potential for GABA channels increased to –70 mV, at which point GABA is inhibitory. These findings imply that either the wiring diagram of frequency control must allow excitatory inputs that reduce the frequency response to CO₂ or GABAergic inputs within the brainstem are inhibitory in some regions of the brainstem at a very young age. Therefore, it is possible that the early decline in ventilatory responsiveness to CO₂ may be mediated by the loss of GABAergic excitation in young rats. However, it is possible that GABA is excitatory in some brainstem sites while inhibitory in others, and it is not clear how such heterogeneity (if it exists) would actually affect the whole animal responses to CO₂.

In summary, new synapses are being formed during the period, when ventilatory responses to CO₂ are changing, and we hypothesize that the development of chemical synapses contributes to the triphasic pattern of ventilatory responsiveness, probably most significantly in the third phase, when ventilatory responses to CO₂ are increasing.

3.3.3. Maturation of excitatory synapses—Just as GABAergic inhibitory synapses are being established, excitatory neurotransmitter-induced currents also undergo changes during the first postnatal month. Electrophysiological recordings from rats aged P0–P3 show that NTS neurons exhibit both glutamatergic and GABAergic currents (Kawai and Senba, 2000). By ages P5–P10, the neurons have differentiated and exhibit either glutamatergic or GABAergic currents; very few neurons display both currents. By P22–P37, neurons show full differentiation, and all of the neurons display either glutamatergic or GABAergic currents (Kawai and Senba, 2000). Early synapse formation may depend on excitatory GABAergic inputs, but glutamate plays a major role in subsequent synapse formation and remodeling (Ben-Ari, 2002). Beyond the neurotransmitter currents elicited, binding affinity of agonists for receptors and receptor density appear to undergo developmental changes. Rao et al. (1997) found that glutamate binding in the NTS increased by 44% between ages P0–P9, decreased by 56% between ages P9–P30, and then stabilized after P30, thereby exhibiting a triphasic pattern. The decrease in glutamate binding between P9–P30 did not involve a change in receptor number but a decrease in receptor affinity for glutamate. Thus, the developmental changes in receptor currents appear to involve changes in both receptor density and receptor affinity, and these changes will be integrated into the larger scheme of synapse formation which goes on at least until P40 in rats. We focused on glutamate and GABA, but the emergence of a variety of excitatory neurotransmitter mechanisms may contribute to the enhanced ventilatory response to CO₂ after P8 in rats.

3.3.4. Differential maturation of neurotransmitter function among central chemosensory sites—We have treated the brainstem as if it were homogeneous with respect to development. This seems unlikely. Messier et al. (2004) administered 8-hydroxy-2-di-*n*-propylaminotetralin (8-OH-DPAT), a 5-HT_{1A} agonist that causes neural inhibition, when bound to pre-synaptic 5-HT_{1A} receptors, focally within the caudal raphe of intact unanesthetized newborn piglets and studied the ventilatory response to inhaled CO₂. Focal dialysis of 8-OH-DPAT into the caudal raphe of piglets age P10 decreased the ventilatory response to CO₂. The ventilatory response to CO₂ was actually enhanced in similarly treated piglets aged P6. There seemed to be an interaction between age and the effect of the 5-HT_{1A} agonist within one CO₂ chemosensory region of the brainstem. The study provides no mechanistic insight into how 8-OH-DPAT modified the response to CO₂ (e.g. no proof that 8-OH-DPAT actually acted on CO₂-sensitive cells within the raphe), but the results indicate that serotonergic mechanisms change as a function of development in the raphe. Similar developmental changes in neurotransmitter processes may occur in other chemosensory sites at other times in development. Such differential timing of maturation of neurotransmitter function among chemosensory sites could contribute to the temporal changes in the ventilatory responses to CO₂ and add a further layer of developmental variation and control on the differences among CO₂ sensory mechanisms and sensitivity that already exist.

3.4. Differential maturation between peripheral versus central chemosensory sites

The carotid bodies make a significant contribution to CO₂ sensitivity (Rodman et al., 2001), and the hypoxic sensitivity of peripheral chemoreceptors is reduced in the early postnatal period, but rises toward adult levels over the first weeks of life. However, CO₂ sensitivity at the level of the carotid body seems to be stable during development (Fig. 3). The CO₂ response of the isolated carotid body is present in young animals and generally equal to the adult response. The multiplicative interaction between hypercapnia and hypoxia increases over the course of development, but the intrinsic CO₂ sensitivity of the carotid body under normoxic conditions does not vary much as a function of age (Pepper et al., 1995; Calder et al., 1997; Bamford et al., 1999). The contribution of peripheral chemoreceptors to the normoxic hypercapnic ventilatory response in intact animals is stable in the neonatal period of development in humans as well, but the interactive effects of CO₂–O₂ at the carotid body seem

to increase as babies mature (Søvik and Lossius, 2004). Thus, we see little evidence that maturation of peripheral chemosensory processes contributes to either the early decline or later rise in CO₂ sensitivity during normoxic testing.

4. Concluding remarks

Neurons from two of the three brainstem chemosensitive regions studied, the LC and NTS, seem to have a fully developed response to hypercapnia at birth that shows no detectable change with development. Based on these findings, the development of chemosensitivity of neurons does not appear to play a critical role in the triphasic developmental pattern of the ventilatory response to hypercapnia seen in intact neonatal rats (Stunden et al., 2001). However, the apparent development of neuronal chemosensitive responsiveness in medullary raphé neurons and the increase in the number of CO₂-sensitive neurons in the lateral reticular nucleus could indicate a role for these neurons in the rise of the hypercapnic ventilatory response after about day P10 (Stunden et al., 2001). It seems possible that astrocytes may modify the chemosensory stimulus or enhance neurotransmitter mechanisms during the period of development, when the ventilatory response to CO₂ rises (phase III), but we lack firm evidence for this hypothesis. The loss of GABAergic excitation may contribute to the initial decline in ventilatory responses to CO₂ (phase I). To the extent that the transition of GABA from an excitatory to an inhibitory neurotransmitter occurs in utero, the early decline in ventilatory responsiveness to CO₂ may be unapparent in precocious species. Finally, synaptogenesis and the emergence of excitatory neurotransmission dependent on glutamate and other excitatory neurotransmitters probably makes a major contribution to the processes generating the fully adult pattern of ventilation (phase III) (Fig. 3).

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Fig. 1.

The early development of the ventilatory response to inspired hypercapnia. A plot of the % increase in ventilation (comparing ventilation while inspiring 5% CO₂ vs. inspiring room air) as a function of the day after birth in neonatal rats. Three phases are evident in the early development: (I) an early ventilatory response to inspired hypercapnia that wanes during the first week of life; (II) a critical window of minimal ventilatory response to inspired hypercapnia; (III) an increase in the ventilatory response to inspired hypercapnia to levels comparable to adults during the second week of life. This figure was drawn based on the data from Stunden et al. (2001); the detailed elements of the pattern may vary in different species.

Fig. 2.

The effect of various protocols for exposure to chronic hypercapnia (CHC) on the development of the ventilatory response to inspired hypercapnia in neonatal rats. The three phases, as described in Fig. 1 are shown with different shading: phase I, gray; phase II, white; phase III, black. The normal pattern of development (CON) is shown below the time line. When exposure to chronic hypercapnia is initiated 5 days before birth and continued until the ventilatory response is tested (CHC-1), the three phases appear but the transition between the phases is right-shifted, i.e. they occur later in development. In contrast, when exposure to chronic hypercapnia is initiated after the rats are born and continued for 7 days (CHC-2), phase III does not occur (up to day P20).

Fig. 3.

The major factors contributing to developmental changes in the ventilatory response to CO₂ are shown above. In the early postnatal period, GABA may cease to be an excitatory ('exc.') neurotransmitter, and excitatory inputs from gap junctions may decline over the entire period of development. Both of these changes may contribute to the early decline in CO₂ responsiveness. The role of peripheral CO₂ chemoreceptors and the neuronal sensitivity to CO₂ among central chemosensory sites seems stable over the course of development. Synaptogenesis and the emergence of excitatory neurotransmitters probably participate in the emergence of adult levels of ventilatory responsiveness to CO₂. Astrocytes proliferate and mature after ~P8, and they may increase neuronal activity in CO₂ chemosensory areas although this hypothesis has not been firmly established.