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The role of CO₂ and central chemoreception in the control of breathing in the fetus and the neonate

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Abstract

Central chemoreception is active early in development and likely drives fetal breathing movements, which are influenced by a combination of behavioral state and powerful inhibition. In the premature human infant and newborn rat ventilation increases in response to CO₂; in the rat the sensitivity of the response increases steadily after ~P12. The premature human infant is more vulnerable to instability than the newborn rat and exhibits periodic breathing that is augmented by hypoxia and eliminated by breathing oxygen or CO₂ or the administration of respiratory stimulants. The sites of central chemoreception active in the fetus are not known, but may involve the parafacial respiratory group which may be a precursor to the adult RTN. The fetal and neonatal rat brainstem spinal-cord preparations promise to provide important information about central chemoreception in the developing rodent and will increase our understanding of important clinical problems, including The Sudden Infant Death Syndrome, Congenital Central Hypoventilation Syndrome, and periodic breathing and apnea of prematurity.

Keywords

central chemoreception; ventilatory response to CO₂; fetal breathing; development

1. Introduction

There is common agreement that central respiratory chemoreception is in place early in development. In this review I will focus on the role of CO₂ and central chemoreception in the control of breathing in the fetus, preterm human infant and full term newborn rat based on the evidence that brainstem development in the newborn rat from P0 until P8-10 is roughly equivalent to that in the third trimester human fetus. From studies of the rate of growth of the brain (Dobbing et al. 1979), functional studies of sleep and arousal (McNamara et al. 1998; Dager et al. 2001; Karlsson et al. 2002; Durand et al. 2004; Blumberg et al. 2005; Seelke et al. 2005), control of breathing (Stunden et al. 2001; Putnam et al. 2005; Wong-Riley et al. 2005; Davis et al. 2006), the autonomic control of heart rate (Hofer et al. 1969), and thermoregulation (Lagerspetz 1966; Conklin et al. 1971; Mouroux et al. 1990), three periods during rat development can be recognized that are roughly comparable to stages of human development: 1) The period from P0 to P8-10, roughly equivalent to the third trimester of pregnancy in the human, 2) a period from P10 to P15

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roughly equivalent to early infancy in the human in which there is dramatic development of the autonomic control of heart rate, sleep, and thermoregulation, and 3) a period from P15 to P30, roughly equivalent to late infancy through young adulthood.

Rats are relatively immature at birth. They have no fur, their eyes are fused and ear ducts are sealed. In some respects they resemble premature infants at 24–26 weeks gestation. However, rats are born with more mature lungs and may not face the same challenges of the very premature infant including surfactant deficiency and poorly developed mechanisms to maintain lung volume. With respect to respiratory control and lung development, the full term newborn rat may be more like a late preterm infant at 32–36 weeks post menstrual age (gestational age + chronological age) (PMA).

Although it is well recognized that the neuronal and muscular components of the respiratory system, as well as the lungs, continue to develop after birth, even when delivered at term, a certain degree of development must occur *in utero* to assure adequate ventilation within minutes of birth. Even very immature human infants have the basic tools to breathe spontaneously and relatively continuously, albeit with significant disadvantages, including a highly compliant chest wall with difficulty maintaining functional residual capacity (FRC), a propensity for airway obstruction, difficulties with coordinating sucking, swallowing and breathing, and immature respiratory control mechanisms resulting in irregular and periodic breathing and apnea.

2. Role of CO₂ in the Control of Fetal Breathing Movements

It is likely that mechanisms designed to maintain oxygenation and acid base balance in the fetus are largely focused on the cardiovascular system and the placenta, since the lungs play no role in gas exchange. On the other hand, a rapid adaptation must take place at birth to survive in a new and different environment that requires the lungs to become the organ of gas exchange immediately after birth. Thus the discovery of fetal breathing movements in the late 1960s generated interest in learning more about the control of fetal “breathing”. Breathing has no gas-exchange function in the fetus and questions about and the role of breathing movements in the transition to extra-uterine life continue remain largely unanswered. However, understanding the mechanisms involved in the regulation of fetal breathing remains important, particularly in advancing our knowledge about the essential changes that must take place just before and immediately after birth and especially the transition from intermittent to continuous breathing. Because the ability to study the human fetus is limited, much of our knowledge has been gleaned from experiments in the fetal lamb.

2.1 Characterization of fetal breathing movements

Fetal “breathing movements” (FBMs), characterized by rhythmic contractions of the diaphragm, intercostals and laryngeal muscles are present in most mammalian species sometime during the second trimester of pregnancy (Jansen et al. 1983; Jansen et al. 1991; Kobayashi et al. 2001). However, the activity of the genioglossus, the major pharyngeal dilator does not appear to be synchronous with that of the diaphragm, unlike what is observed in infants and adults (Johnston et al. 1988). FBMs characteristically occur during periods of low voltage, high-frequency electrocorticogram (LV-ECoG), rapid eye movements and hypotonia, similar to many features of REM sleep (Dawes et al. 1972). During periods of high voltage low-frequency ECoG (HV-ECoG), the fetus is characteristically apneic. Although controversial, the fetus may spend a very small amount of time in a more active “awake” state characterized by LV-ECoG, fetal breathing, increased tonic and phasic nuchal EMG activity, and increased mean arterial blood pressure. In the fetal lamb FBMs are irregular with an abrupt beginning and ending of diaphragmatic (DIA)

EMG electrical activity. There is no evidence for a gradual decrease in DIA EMG activity representing expiratory braking frequently observed in the premature human infant (Harding et al. 1980; Dawes et al. 1982; Rigatto et al. 1986). FBMs generate a negative tracheal pressure change of ~2–5 mmHg and on average have a T_I of 0.45 s, T_E of 0.74 s and T_{TOT} of about 1.12 s (Dawes et al. 1982; Rigatto et al. 1988). The incidence of FBMs appears to increase after meals and is associated with increased plasma glucose levels. It has been suggested that the discovery of the intermittent nature of FBMs and their relationship to state may be the most important contribution to fetal physiology of the century (Rigatto 1996) and the explanation for the intermittent nature of fetal breathing continues to be incomplete. In the fetal lamb the incidence of FBMs increases and becomes more episodic during development reaching about 30% by term and characteristically decreases during the 5 days prior to the onset of labor (Berger et al. 1986).

2.2 Central control of fetal breathing

Development of the instrumentation in the 1960s that allowed physiologic measurements to be made in the unanesthetized fetal preparation (Meschia et al. 1965) was an important first step in studying mechanisms that control fetal breathing. Fetal breathing responds to chemical stimuli and other agents that have been found to affect postnatal breathing. Increasing P_{aCO_2} increases fetal breathing as evidenced by an increase in tracheal pressure, DIA EMG activity, and frequency (Moss et al. 1979; Dawes et al. 1982; Rigatto et al. 1988), but only during LV-ECOG. However, breathing during HV-ECOG can be stimulated by unphysiologically high levels of CO_2 or when pH is low (Molteni et al. 1980). External cooling is also a powerful stimulant of fetal breathing, and unlike CO_2 , results in breathing during both low and high voltage ECOG states (Gluckman et al. 1983; Moss et al. 1983; Kuipers et al. 1997). Moreover, administering CO_2 during cooling enhances breathing during both states (Kuipers et al. 1997). A number of excitatory and inhibitory neuromodulators have also been implicated in controlling fetal breathing, including adenosine, GABA, opioids, prostaglandins, serotonin, progesterone, substance P, TRH, and catecholamines (Moss et al. 1989; Wong-Riley et al. 2005).

Both hypoxia and hypercapnia increase electrical activity in the carotid sinus nerve demonstrating that carotid chemoreceptors are active in the fetus (Blanco et al. 1984). Changes in heart rate (f_H) in response to CO_2 and hypoxia are also thought to be mediated by peripheral chemoreceptors. Both hypoxia and hypercapnia produce substantial decreases in f_H . In the case of hypoxia, the change in f_H is inversely related to the resting oxyhemoglobin saturation (Boekkooi et al. 1992). There are several lines of evidence, however, that suggest that central mechanisms drive fetal breathing. Central chemoreceptors appear to be present within days after birth in the rabbit (Wennergren et al. 1983) and piglet (Wolsink et al. 1991) suggesting their previous presence in the fetus. Decreasing P_{aO_2} inhibits rather than stimulates fetal breathing (Koos et al. 1987; Jansen et al. 1991), the inhibition likely originating from a region in the upper lateral pons (Johnston et al. 1989; Johnston et al. 1993). Moreover, denervation of the carotid bodies does not alter fetal breathing, fetal state, or the stimulating effect of CO_2 (Koos et al. 1987; Moore et al. 1989). In addition, alteration of bicarbonate concentrations in the CSF can stimulate or depress fetal breathing in the chronically instrumented lamb (Hohimer et al. 1983). However, bilateral surface lesions in on the ventral surface of the medulla corresponding to the classical Area “S” lesions, although reducing the incidence of FBMs for several days after the lesion, do not affect the FBM response to CO_2 (Jansen et al. 1993).

2.2.1 Possible sites of central chemoreception in the fetus—The evolving concept of multiple brainstem sites for central chemoreception (Nattie et al. 2009) should be taken into consideration in the interpretation of lesion experiments such as those described

above, as there are several candidate sites for central chemoreception in the fetus including the parafacial respiratory group (pFRG), nucleus tractus solitarius (NTS), locus coeruleus (LC), and the medullary raphe (MR). For example, the serotonin (5-HT) system is one of the earliest neurotransmitter systems to develop (Herlenius et al. 2001; Kinney et al. 2007) and 5-HT neurons in the medullary raphe are sensitive to CO₂, are located near a rich blood supply, and could be an important modulator of fetal breathing during fetal life (Corcoran et al. 2009). Administration of 5-hydroxytryptamine to the fetus stimulates breathing (Quilligan et al. 1981) and importantly, endogenous serotonin modulates fetal respiratory rhythm in fetal reduced preparations (Di Pasquale et al. 1994).

Although 5-HT can stimulate breathing in the fetus, whether 5-HT neurons are chemosensitive during early development is unknown. There is evidence in the rat that the role of 5-HT neurons in chemoreception may become more important later in development based on experiments showing that the chemosensitivity of 5-HT neurons in slices and culture parallels the increase in the hypercapnic ventilatory response that occurs after about P12 (Wang et al. 1999; Corcoran et al. 2009). In addition, in adult animals, the activity of 5-HT neurons is state dependent, having the highest level of activity during wakefulness, a lower level of activity during NREM sleep and almost no activity during REM. It is unknown whether this state dependence of 5-HT neuronal activity is present during early development. In the fetus, breathing only occurs during the REM-like LV-ECoG state. Furthermore CO₂ stimulates breathing only during this state, unless accompanied another stimulus such as cooling. If, as in adults, 5-HT neurons have only limited activity during REM, it is unlikely that they would contribute heavily to central chemoreception, but may provide important state related inputs to other central chemoreceptor sites. This input could be excitatory or possibly inhibitory depending on which receptors were activated. Similarly, the LC might provide a state related drive to breathing. Thus, although central chemosensory activity is present in the fetus, the exact locations or combination of sites involved remains unknown.

2.2.2 Fetal Brainstem-Spinal Cord Preparations—Recent information about the development of neural circuits responsible for fetal breathing has come from studies in *in vitro* fetal rodent models (Greer et al. 2006). Based on *in vitro* electrophysiological recordings in the isolated rat fetal brainstem-spinal cord (Di Pasquale et al. 1992; Greer et al. 1992), and ultrasound recordings of the rat fetus *in utero* in anesthetized dams (Kobayashi et al. 2001), it appears that respiratory rhythmogenesis first emerges around E16.7-17. In ultrasound recordings, respiratory activity begins as single isolated movements progressing to episodes of more clustered events. Unlike breathing in the conscious fetus, the burst patterns from *in vitro* preparations are continuous, perhaps due to the lack of descending inhibitory influences in these preparations. The episodic nature of fetal breathing movement in conscious intact mammals remains an enigma and the mechanisms remain unknown.

2.3 Role of inhibition in the control of fetal breathing

2.3.1 Inhibitory role of the upper lateral pons—Powerful inhibitory mechanisms active in the fetus, superimposed on state related cycles of excitation and inhibition, may be responsible for the episodic nature of fetal breathing. Based on transection and lesion experiments, it has been postulated that regions located in the upper lateral pons contribute to the inhibition of fetal breathing during hypoxia (Gluckman et al. 1987). In addition, lesions in this region that reverse the breathing depression caused by hypoxia also allow CO₂ to stimulate breathing during the HV-ECoG state (Johnston et al. 1989) suggesting that this region may also provide some level of tonic inhibition, especially during periods of HV-ECoG. Further evidence for the presence of a fetal lateral pontine inhibitory area comes from transection and lesion studies in newborn animals showing that the depressive phase of

the “biphasic” hypoxic response can be attenuated by mid-collicular but not pre-collicular transections, and red nucleus lesions in rabbits and rats (Martin-Body et al. 1988; Waites et al. 1996).

2.3.2 Inhibitory role of adenosine and other factors—Adenosine has been suggested as an important source of inhibition of FBMs in the fetus. Activation of adenosine A(2A) receptors appears to contribute to hypoxic inhibition of fetal breathing (Koos et al. 1994; Koos et al. 2002). Elevated levels of adenosine, presumably related to the relative hypoxic fetal environment, may also be a source of tonic inhibition by providing inhibition of respiratory drive via A(1) receptors and inhibiting the REM-like behavioral state via A(2A) receptors (Koos et al. 2001). Antagonists of adenosine receptors such as caffeine stimulate fetal breathing movements. Moreover, blocking adenosine A(1) receptors reverses ethanol-induced inhibition of fetal breathing movements (Watson et al. 1999).

In addition to the inhibitory actions of adenosine, both GABAergic and glutamatergic mechanisms appear to be important in suppressing the rarely observed “awake” state characterized by LV-ECOG, increased nuchal tone, increased blood pressure, and fetal breathing. For example, administration of NMDA antagonists increases the incidence of this “awake” state, which is prevented by increasing extracellular GABA by inhibiting reuptake mechanisms (Bissonnette et al. 1995). The role of other inhibitory neuromodulators such as opioids may also contribute to inhibition (Moss et al. 1989; Herlenius et al. 2001). Moreover, since cooling is also a potent stimulator of fetal breathing, the normal warm temperatures of amniotic fluid that bathe peripheral skin thermo-sensors may provide a relative level of tonic inhibition.

2.3.3 Inhibitory role of the placenta—The placenta may also be a source of tonic inhibition of breathing in the fetus, which then disappears when the fetus is separated from the placenta at birth. This mechanism may also be involved in the maintenance of continuous breathing after birth (Adamson et al. 1991; Alvaro et al. 1993) but is likely not the source of the initiation of breathing (Kuipers et al. 1992). The presence of some inhibitory placental factor is also supported by experiments showing that injection of a placental extract into the carotid artery of fetal sheep inhibits breathing induced by previous umbilical cord occlusion (Alvaro et al. 1993). Although the exact nature of such a placental factor remains unknown, prostaglandin E₂, the level of which is known to decrease after birth and after cord occlusion, remains a likely candidate (Alvaro et al. 2004). An inhibitory role for prostaglandins is further supported by experiments in fetal lambs showing that the prostaglandin synthetase inhibitor, meclofenamate, stimulates and prostaglandin E₂ inhibits fetal breathing movements independent of glucose concentration (Murai et al. 1984).

2.4 Role of sleep or behavioral state in fetal breathing

Fetal breathing is stimulated or inhibited in association with behavioral state. It is possible that increasing levels of state related excitatory influences on breathing are partially achieved by altering the sensitivity of central chemoreception as suggested by Guyenet, et al. (Guyenet et al. 2009). According to this hypothesis putative chemoreceptors receive increasing excitatory inputs, possibly stress related, from neuronal networks with state related activity such as MR 5-HT neurons, LC noradrenergic (NA), or possibly orexinergic neurons, which in turn lower the CO₂ threshold and thus contribute to increasing respiratory drive. It is unknown, however, whether MR 5-HT and LC NA neurons have state related activity in the fetus. However, if their activity patterns are similar to adults, both MR 5-HT neurons and LC NA neurons would be expected to be relatively quiescent during REM and active during wakefulness; however both states are associated with breathing in the fetus. An excitatory contribution from the MR and LC may therefore seem reasonable during

wakefulness (rarely seen), but less likely during REM. Thus the source of possible excitatory input to breathing during REM remains unknown. Breathing might also be actively inhibited during HV-ECoG since there are many sources of inhibition, but a link between HV-ECoG and any particular source of inhibition has not been identified.

Superimposed on this state related drive is a changing level of tonic inhibition possibly from placental factors including prostaglandins, relatively high concentrations of adenosine related to the hypoxic state of the fetus, and the upper lateral pons. Thus with increasing levels of inhibition, such as during hypoxia or after the administration of narcotics, any state related excitation could be overridden causing all FBMs to disappear. In contrast, decreasing levels of inhibition, as might occur at birth or after cooling, might be the origin of more continuous breathing which could occur both during LV-ECoG and HV-ECoG states. Figure 1 illustrates the possible relationships between state related intermittent breathing activity and varying levels of tonic inhibition.

2.5 Control of breathing in the human fetus

While we have benefited from the study of respiratory control in the fetal lamb and rat, little specific information is known about respiratory control in the human fetus. Human fetuses have episodic rapid irregular breathing movements similar to those observed in other mammals. Also, similar to what is observed in fetal lambs, FBMs occur about 30% of the time when observed with real-time ultrasonic scanners. In late preterm fetuses (34–35 weeks gestation), FBMs are stimulated after meals and are correlated with maternal plasma glucose levels (Patrick et al. 1978). The FBM response to glucose has been observed as early as 24 weeks gestational age (GA) (Nijhuis et al. 1986) and increases with advancing gestation (Harper et al. 1987). In addition, there are sustained increases in FBMs between 0100 and 0700 that are not related to plasma glucose concentrations and may be circadian rhythm related. During labor the incidence of FBMs decreases dramatically and they are no longer stimulated by glucose administration (Boylan et al. 1980). Maternal coffee drinking can cause a doubling in the increase in FBMs and a decrease in baseline fetal f_H (Salvador et al. 1989), whereas maternal ETOH and narcotics suppress FBMs. There are limited data about brainstem sites in the human fetus that might be involved in central chemoreception. In an important study, the anatomic relationships of the nucleus tractus solitarius (NTS) to other regions thought to be important for respiration were studied in the human fetus using the bidirectional lipophilic fluorescent tracer, DiI. Labelling was found in several medullary areas involved in autonomic and respiratory control, including stellate cells in the caudal raphe and cells and fibers in the Dorsal Motor Nucleus (DMN) of the vagus, the nucleus ambiguus complex, the rostral and caudal ventrolateral medulla and the medullary reticular formation. This pattern was established by mid-gestation and was consistent with anatomic patterning in animals. An important finding was that of stellate cells at the junction of the nucleus raphe pallidus and the arcuate nucleus at the ventral medullary surface which project to the NTS. These could be homologous to chemosensitive 5-HT neurons found in the midline raphe in rodents (Zec et al. 1997).

3. The premature human infant and the newborn rat

3.1. Breathing irregularity in the premature human infant and newborn rat

3.1.1 The premature human infant—Breathing in premature human infants is characterized by irregularity, periodic breathing, and frequent apnea. Breathing irregularity is largely dependent on sleep/behavioral state and is most common in active or REM sleep (AS), whereas breathing is more regular during quiet or NREM sleep (QS). In the human infant from 30 to 40 weeks PMA, breathing irregularity appears to increase slightly from 30 to 36 weeks and then steadily decreases. Breathing is irregular approximately 60–70% of the

time at term and doesn't decrease below 50% until approximately 3 months of age (Parmelee et al. 1972). The changes in the amount of irregular breathing generally correlates with the changes in the percentage of AS that occurs from 30 weeks to term (Mirmiran et al. 2003). Figure 2 shows the percent of irregular breathing closely matching the amount of active sleep in the premature infant.

3.1.2 The newborn rat—Full term neonatal rats also exhibit breathing irregularity, often associated with breathing pauses and acute decreases in f_H (bradycardia) (Cummings et al. 2009a). This is most pronounced immediately after birth, becoming more regular by P10, and appears to be independent of vagal or supratentorial inputs (Zhou et al. 1996). In the experiments of Zhou, et al, performed in decerebrate and vagotomized newborn rats, adding CO_2 to the breathing gases increased phrenic amplitude and minute activity with little effect on the timing variables or the degree of variability. Studies in conscious rat pups studied in their own nests with their siblings have shown that after a regular respiratory pattern is established (P10-P12), bilateral denervation of the carotid sinus nerve causes a reversion to an irregular pattern. These findings suggest that increasing strength of excitatory carotid body reflexes participates in the transition from an irregular to a regular pattern of breathing (Hofer 1986).

Moreover, the degree of irregularity is dependent not only on behavioral state, but also on temperature (Cameron et al. 2000) and is further modulated by the level of hypoxemia (Cameron et al. 2000) and hypercapnia (Cummings et al. 2009b). Interestingly, whether temperature is rising or falling also appears to play a role (Cameron et al. 2000). Measures of respiration, including $\dot{V}_T$, T_I , T_E , and $\dot{V}_E$, in P4 rat pups are considerably more variable in a warm environment (36 °C) compared a cool environment (24 °C) and the differences are greatest when exposure to a warm environment precedes exposure to cold. Interestingly hypoxia decreases the variability and largely abolishes any effect of temperature. Cummings and Frappell reported that the increasing variability associated with a warm environment was also associated with an increased immediate $\dot{V}_E$ response to CO_2 (Cummings et al. 2009b). Taken together, these data are consistent with observations in human premature infants that mild cooling regularizes breathing and infants kept in warmer environments exhibit more unstable breathing and apnea (Daily et al. 1969; Perlstein et al. 1970).

3.2 Periodic breathing in the premature human infant

In addition to breath-to-breath variability, human premature infants often exhibit respiratory instability in the form of periodic breathing (PB). Often the terms PB and irregularity are used interchangeably causing some confusion since the mechanisms responsible, although interrelated, are most likely different. In contrast to irregularity where there is characteristic breath-to-breath variability, PB has been defined as alternating periods of breathing and apnea. Indeed, in the human infant, most apnea of prematurity is associated with PB. For study purposes PB has been commonly defined according to Kelly et al, as three or more sequential apneas lasting more than 3 seconds, separated by periods of breathing with a duration of < 20 seconds (Kelly et al. 1979). The incidence of PB and apnea is greatest in the most immature infants and gradually decreases from 30 to 40 weeks PMA (Parmelee et al. 1972) and by 43 weeks PMA, the incidence of PB and apnea approaches that of the full term infant (Ramanathan et al. 2001). The degree of PB can be quantified by measuring the periodic cycle duration (PCD) which is the interval from the initial breath in one cluster of breaths to the initial breath in the next cluster and which takes into account both apnea and breathing cluster duration. In a study of both term and preterm infants, Wilkinson et al reported a decrease in PCD during development. Figure 3 is a plot of the PCD of both preterm and term infants plotted against PMA in days (Wilkinson et al. 2007).

3.2.1 The apneic threshold and periodic breathing—Periodic breathing can be induced by hypoxemia or respiratory depressants and alleviated by respiratory stimulants, including caffeine, and breathing oxygen or low levels (0.5–1%) of CO₂. Sleep has long been known to favor the appearance of PB. In premature infants, breathing cycles between PB and non-PB breathing in both QS and AS. It has been suggested that a major factor producing PB during sleep relates to a decrease in ventilation, decrease in the sensitivity to CO₂, and a decrease in arterial O₂ tension (Rigatto et al. 1972). The apneic threshold has been determined to be much closer to eupnic levels of PaCO₂ in premature infants compared to adults (Khan et al. 2005). Thus the combination of greater oscillations in PaCO₂ and a decreased CO₂ sensitivity may allow PaCO₂ to frequently decrease below apneic threshold and contribute to the apnea associated with PB. Figure 4 illustrates the difference between premature infants and adults with respect to the proximity of eupneic PaCO₂ and apneic threshold.

3.2.2 Contribution of loop gain to periodic breathing—Periodic breathing can also be examined in terms of the engineering concept of loop gain (Khoo et al. 1982; Khoo 2000). For any negative feedback system, a disturbance (u), which increases alveolar ventilation ($\dot{V}_A$) by $\Delta\dot{V}_A$ from some steady-state equilibrium level, will lower PaCO₂ by ΔPaCO_2 and thus evoke a negative corrective action (e) to suppress the disturbance. The magnitude of corrective action comes from a combination of the gain of the controller (for example, the carotid body and/or central chemoreceptors), and the gain of the lungs and respiratory muscles, termed “plant” gain. The ratio of e/u defines the system loop gain. The other essential feature that favors instability of the system is the time delay between the initial change in $\dot{V}_A$ caused by the disturbance to the corrective change in $\dot{V}_A$ produced by the controller and plant. Thus, for an intermittent disturbance such as a sigh producing an abrupt decrease in PaCO₂, when the loop gain is high (the response is high with respect to the initial change) and the response is 180° out of phase with the perturbation, a sustained oscillation can result. In premature infants, the level of arterial oxygen tension is frequently in the 40–70 range, lower than that of adults. After the first few days of life carotid chemoreceptor drive at rest may be a significant component of the overall drive to breathe (Rigatto 2003) and contribute to respiratory instability. This is supported by reports of a positive relationship between apnea frequency and the strength of the carotid body reflex (Nock et al. 2004; Cardot et al. 2007). Moreover, the low FRC in the premature infant may result in decreased “buffering” of changes in ventilation. Thus both increasing carotid body gain and low FRC may be significant contributors to an increased loop gain in the developing infant. Interventions aimed at increasing FRC, including prone positioning (Martin et al. 1979; Wagaman et al. 1979; McEvoy et al. 1997), a negative pressure applied around the chest (Thibeault et al. 1967), and continuous positive airway pressure (Kattwinkel et al. 1975) result in a decrease in periodicity. In this regard the application of CPAP has been shown to reduce loop gain in lambs breathing periodically (Edwards et al. 2009). Since an increase in peripheral chemoreceptor gain is most likely necessary to increase loop gain in the immature system, this might explain why periodic breathing does not occur immediately after birth but begins after several days. This is consistent with carotid body becoming relatively silent immediately after birth and resetting to a higher gain over the first few days of life (Blanco et al. 1984).

3.2.3 Contribution of sleep to periodic breathing—Unlike irregular breathing which is mostly related to stage of development and behavioral state (modulated by temperature and the degree of hypoxia and hypercapnia), PB occurs in both QS and AS. During PB in AS breathing is very irregular and during PB in QS breathing is more regular (Rigatto 2003). Sleep could also promote PB with further reductions in FRC and increases in chest wall compliance, particularly during AS (Knill et al. 1976; Henderson-Smart et al. 1979). In

premature infants decreases in FRC have been observed during PB (Thibeault et al. 1967) and are often associated with decreases in oxyhemoglobin saturation.

3.2.4 Sighs, obstructed breaths, and periodic breathing—Augmented breaths or sighs are common in premature infants and are more frequent in AS compared to QS and most likely serve to increase lung volume and maintain FRC. The mechanisms responsible for sighs have not been clearly elucidated but are likely related to changes in irritant and chest wall reflexes caused by a decrease in lung volume, and particularly in the neonate, asphyxia and hypoxemia (Bartlett 1971; Thach et al. 1976; Alvarez et al. 1993). During PB, sighs appear to be distributed roughly equally both before and after respiratory pauses (Alvarez et al. 1993; Weintraub et al. 1994). When occurring after the pause, sighs almost always appear within the first 1–2 breaths, suggesting a decreased lung volume occurring during the pause (Henderson-Smart et al. 1979; Poets et al. 1997). In term infants sighs have also been observed to trigger episodes of PB, presumably associated with a drop in PaCO_2 below apneic threshold producing an initial respiratory pause (Rigatto 2003) in combination with high loop gain and response delay.

Obstructed breaths often accompany otherwise central apnea, especially those of longer duration and the combination is often termed “mixed” apnea (Milner et al. 2004). Obstructed breaths can also occur during PB, but the pattern may differ from those occurring with longer apneas. Based on measurements of airway resistance, Miller et al reported complete airway obstruction at the level of the pharynx occurring at the beginning of each respiratory phase and continuing for approximately one-third of the breathing cycle (Miller et al. 1988). Using another approach which involves the presence or absence of a magnified cardiac oscillation signal present in the respiratory flow tracing, Lemke et al determined that during PB, in almost all cases, the magnitude of the cardiac oscillation remained relatively unchanged during the apneic phase of the cycle (Lemke et al. 1996). These data, taken together, indicate that during the relative short apneas associated with PB, the apneic phase is largely unobstructed but airway obstruction frequently occurs during the first third of the respiratory phase.

3.3 Periodic breathing in the newborn rodent

Although common in premature human infants, classic PB is rarely observed in unmanipulated newborn or adult rodents, whereas breath to breath variability (irregularity) is commonly seen. PB can be induced in adult mice of certain strains, however, including the heterozygous $\text{Mecp2}^{+/-}$ (Bissonnette et al. 2008), $c\text{-ret}^{+/-}$ (Aizenfisz et al. 2002) and inbred B6 (Han et al. 2002) mouse during the recovery phase (air or 100% O_2) after exposure to hypoxia or asphyxia. Figure 5 shows the development of a periodic breathing pattern after exposure to hypoxia in the heterozygous $\text{Mecp2}^{+/-}$ mouse, a genetic model for Rett Syndrome in humans in which PB often occurs during sleep. The reason for the absence of periodic breathing in the newborn rodent is not known but may be related to the relative insensitivity of peripheral chemoreceptors or perhaps better mechanisms to maintain FRC (low loop gain), relatively short delay between the initial change and resulting response, or a lack of proximity of eupneic PaCO_2 to apneic threshold.

3.4 The ventilatory response to CO_2 in the human preterm infant

Inhalation of CO_2 results in an increase in $\dot{V}_E$ even in the most premature human infants. In humans, the full term infant has a ventilatory response slope ($\Delta\dot{V}/\Delta\text{PaCO}_2$) comparable to that of the adult, whether steady state or rebreathing methods are used (Avery et al. 1963), but the position of curve is to the left of that of the adult and shifts to the right with increasing age reflecting the lower eupneic PaCO_2 in the infant compared to the adult. In the premature infant, the slope of the response to CO_2 , measured as $\Delta\dot{V}/\Delta\text{PaCO}_2$ increases with

maturation (Krauss et al. 1975; Rigatto et al. 1975a; Frantz et al. 1976; Krauss et al. 1976). Unfortunately, most studies report on only two gestational age ranges in relatively mature preterm infants over a range of 30–37 weeks; there is little information on more immature infants < 30 weeks. Early studies suggested that the ventilatory response to CO₂ in the preterm infant consisted of changes in both respiratory frequency (f_R) and $\dot{V}_T$ (Cross et al. 1953), thought to be explained by changes in the vagally mediated Hering-Breuer (HB) reflex during development. Bodegard reported a shift in the pattern of the CO₂ response to steady state exposure of 4% CO₂ from one in which there was an abrupt increase in respiratory frequency f_R without much change in $\dot{V}_T$ early in prematurity to one in which there was a gradual increase in tidal volume, followed by a f_R increase accompanied by a further increase in tidal volume. This was interpreted as a shift from a strong inspiratory inhibitory reflex (the immediate rate increase) during prematurity to a weaker reflex more consistent with that observed in adults (Bodegard 1975).

Later studies using similar steady state techniques showed that once stabilized, the changes in $\dot{V}_E$ after CO₂ breathing in premature infants are largely secondary to changes in $\dot{V}_T$ with little change in f_R (Rigatto et al. 1975a) and this continues to be reflected in most reviews. If one considers the entire response, however, there is evidence for a transient increase in f_R (1–2 minutes) followed by a gradual decrease to baseline levels. The initial increase in f_R is associated with a decrease in T_E , not T_I as might be expected if the increase in f_R were due to activation of the inspiratory inhibitory reflex (Martin et al. 1985; Noble et al. 1987). After the initial increase there is a decrease in f_R to baseline values associated with lengthening of T_E . It is likely that the initial ventilatory response to CO₂ is more related to peripheral chemoreceptor CO₂ sensitivity which may be relatively mature at birth.

3.4.1 Presence of expiratory braking in the ventilator response to CO₂—The lengthening of T_E over the last 5–6 minutes of CO₂ exposure is often associated with expiratory braking and in some infants, audible grunting. The first reports of expiratory braking during CO₂ exposure in premature infants were later supported by studies of Eichenwald et al (Eichenwald et al. 1993) who measured $\dot{V}_E$ and diaphragmatic (DIA) and posterior cricoarytenoid (PCA) muscle EMG activity in 33–34 week gestation infants at 3–4 days of age. They concluded that exposure to steady state CO₂ resulted in activation of both the diaphragm and the PCA and that the response to CO₂ was associated with an increase in T_E characterized by expiratory braking. An examination of the changes in diaphragm and PCA EMG activity and airflow revealed that most of the infants experienced prolonged post-inspiratory DIA activity which was highly correlated with increases in T_E , despite increases in PCA activity. In some infants there was also evidence for laryngeal braking. The expiratory braking and prolongation of T_E observed in premature infants is in marked contrast to what is observed in older infants and adults where there is a decrease in airway resistance during both inspiration and expiration and an increase in respiratory frequency characterized by a shortening of T_E (Avery et al. 1963; Clark et al. 1972; Bartlett 1979).

3.4.2 Etiology of the decreased ventilatory response to CO₂ in immature infants—To help answer the question of whether the diminished response in the more immature infants is secondary to a diminished central “sensitivity” to CO₂ or whether mechanical or vagally mediated reflexes play a role, Franz et al measured both the $\dot{V}_E$ and the peak pressure response to CO₂ in two groups of preterm infants (29–32 wks and 33–36 wks) on day 3 and 10 of life. Similar to the findings of other investigators, they found a lower slope of the $\dot{V}_E$ response to CO₂ in the more immature infants that was correlated with peak pressure responses. Some immature infants had only a minimal ventilatory response to CO₂ and were observed to have audible “grunting” and prolongation of T_E . These infants, however, had normal peak pressure responses, indicating that their diminished responses were likely due to mechanical factors such as the requirement to maintain lung volume by

prolonging expiratory time (Frantz et al. 1976). The mechanisms responsible for the apparent increase in CO₂ sensitivity with postnatal maturation independent of changes in the mechanical properties of the lung remain unclear. Possibilities include: 1) developmental enhancement of the integrative function on the central nervous system respiratory controllers, 2) an increase in the sensitivity of the central and/or peripheral chemoreceptors to CO₂ and/or [H⁺], 3) change in the mix of neurotransmitters and receptors, or 4) a maturational change in the effectors of the respiratory system including diaphragm and intercostal muscles (Guthrie et al. 1980). In support of the last possibility, Keens has reported an increase in the percentage of type I fibers (low fatigability) in the diaphragm and intercostals muscles of human infants with advancing gestational and postmenstrual age (Keens et al. 1978).

3.4.3 Interactions between the level of oxygenation and the ventilatory response to CO₂

—The interactions between the level of oxygenation and the steady state ventilatory response to CO₂ also appear to be different from those in adults. In the adult, the sensitivity of the ventilatory response to CO₂ increases with decreasing levels of PaO₂; the response appears to be the opposite in the preterm infant, with the ventilatory response to CO₂ increasing with increasing PaO₂ (Rigatto et al. 1975b). However, one must take into consideration that most evaluations of CO₂ chemosensitivity in infants are done using a steady state technique where measurements are often taken after 5–10 minutes of exposure. The ventilatory response to hypoxia is biphasic in the preterm infant; thus measurements of the response to CO₂ are most often made after there is considerable central ventilatory depression from hypoxia. However, at least one study in preterm infants suggests that CO₂ interacts positively with hypoxia (positive CO₂/O₂ interaction) at the peripheral chemoreceptor level (Albersheim et al. 1976). It is possible that during longer exposures to hypoxia and CO₂ that central ventilatory depression associated hypoxia might override the positive CO₂/O₂ interaction at the peripheral chemoreceptor (Wolsink et al. 1992). There also may be considerable species differences that might affect the balance and timing between peripheral and central chemosensitivity to CO₂. The degree of “roll off” during steady state exposure to hypoxia may provide some clues. In the human preterm infant, for example, the roll off is extreme often producing apnea after 4–5 minutes of exposure. This roll off is not as extreme in the newborn rat or piglet and in these species there is a positive CO₂/O₂ interaction similar to the adult, except during very severe hypoxia (Saetta et al. 1987; Wolsink et al. 1992).

3.5 Ventilatory response to CO₂ in the newborn rat

The steady state ventilatory responses to CO₂ in more mature newborn animals such as the piglet or the goat have been reported to be similar to adults although few direct comparisons have been performed (Lowry et al. 1996; Putnam et al. 2005). Ventilatory responses to CO₂ in the newborn rat, however, in many respects are similar to the human premature infant. Studies that have examined the response to CO₂ in the first few minutes have shown a transient increase in f_R (Cummings et al. 2009b). After equilibration, the changes in V_E are almost entirely secondary to changes in V_T (Stunden et al. 2001; Davis et al. 2006). In contrast to what is commonly observed in human premature infants, expiratory braking is not as evident in young rats, although in the first week of life, in response to CO₂, there is also a decrease in f_R and a prolongation of T_E. Moreover, the lengthening of T_E appears to be influenced by GABAergic mechanisms, since it is abolished by pretreatment with bicuculline, a GABA_A receptor antagonist (Abu-Shaweesh et al. 1999). Similar to more mature infants, children and adults, f_R commonly increases in response to CO₂ in adolescent and adult intact rats (Abu-Shaweesh et al. 1999).

Resting ventilation and the ventilatory response to CO₂ in the newborn rat is also affected by body and environmental temperature and this is age dependent. P2-P3 and P5-P6, but not P8-P9 rat pups, have a higher resting ventilation at body temperatures of 32–33°C compared to 36–37°C or 38–39°C. There is some controversy about the effect of temperature on the $\dot{V}_E$ response to CO₂; some have observed greater overall responses at cooler temperatures, but only in the P2-P3 and P5-P6 rat pups (Malcolm et al. 1996), whereas others have noted greater immediate responses at warmer temperatures (Cummings et al. 2009b) in these same age ranges. The differences in these observations may be partially due to the focus on the immediate (likely peripheral chemoreceptor) responses by Cummings et al and the measurement of a more steady state response by Malcolm et al.

The effect of oxygenation on the steady state ventilatory response to CO₂ in the newborn rat is also different from that in the premature human infant and shows a positive correlation between hypoxia and the response to CO₂ (Saetta et al. 1987). These differences may be secondary to the combination of the species and age related relative contribution of peripheral and central chemoreceptors to the total ventilatory response to CO₂ and to varying degrees hypoxic ventilatory depression.

3.5.1 Changes in the sensitivity of the ventilatory response to CO₂ with advancing age in the rodent—In the young rat, the sensitivity of the steady state ventilatory response to CO₂ generally increases with age. There is general agreement that the response increases between P12-P15 and P30, but there is considerable debate over the change in the response in the first few days of life. Some have contended that the response is strong immediately after birth but decreases, reaching a nadir by about P8-P12, followed by a steady increase until about P30 (Serra et al. 2001; Stunden et al. 2001; Putnam et al. 2005). Others have suggested that the response is relatively constant over the first few days until P8-12, and then increases steadily until P30 (Davis et al. 2006). A nadir in the ventilatory response to CO₂ at P8-12 could represent a “critical period” of development which correlates with an abrupt decrease in glutamate and increase in GABA content in regions of the developing brain stem important for respiratory control (Wong-Riley et al. 2005). This concept is also attractive because it may correlate with the highest incidence of The Sudden Infant Death Syndrome (SIDS) in humans at around 2–3 months of age, the etiology of which is thought to be related to abnormalities in respiratory and autonomic control during a critical period associated with changing control mechanisms.

Some have argued that the ventilatory response to CO₂ changes little over the first 10 days of life and have suggested that one contributing factor to the difference in results is the different methods used to express CO₂ sensitivity, one normalized to body weight and the other as a percent of eupnic ventilation (Davis et al. 2006). Moreover, Stunden et al (Stunden et al. 2001) used “head-out” pressure plethysmography and defined sensitivity as the slope of the response (for example, $\Delta\dot{V}_E/\Delta\%CO_2$) calculated from exposing each animal to several concentrations of CO₂, whereas Davis et al (Davis et al. 2006) used flow through whole body plethysmography and calculated sensitivity from a single measurement of ventilation after 3–5 minutes of breathing 7% CO₂. Both of these methods have their limitations that may have contributed to different results. Figure 6 summarizes the changes in the ventilatory response to CO₂ with age using different methodologies. Most agree that the ventilatory response to CO₂ increases after P12-15, but there remains uncertainty about the course of the response during the first 10 days of life.

The large variation in the individual and daily measurements of the ventilatory response to CO₂ may also be influenced by how the experimental methodology takes into account changes in growth and development that occur during the first few days of life. For example, there is a doubling of body weight from P2 to P8 accompanied by changes metabolic rate

(Taylor 1960; Mortola 2001), the ideal thermal environment (Mortola et al. 1998), sleep and activity cycling (Blumberg et al. 2005), and interactions with the dam and siblings. Ideally studies should be performed in the nest with the siblings and dam. This is rarely practical but some investigators have made measurements of heart rate and respiratory frequency in this environment (Hofer et al. 1969; Hofer 1986).

3.5.2 The importance of thermoregulation in respiratory measurements in immature rodents—The “head out” plethysmograph is commonly used to make respiratory measurements in small rodents. A commonly used technique is to warm the “body” portion of the chamber to maintain body temperature in some range. Often, however, little attention is made to controlling the temperature that is flowing around the head. It is well known that the trigeminal area of the face is exquisitely sensitive to temperature and cooling the face can increase metabolic rate even if abdominal skin and core temperature are normal (Mestyan et al. 1964). Figure 7 shows increases in metabolic rate associated with facial cooling in a premature human infant in the presence of a stable rectal and abdominal skin temperature. The thermoneutral environment also changes with growth and maturity, so that the environmental temperature designed for the P10 animal may not be warm enough for the younger pup. Similarly, a thermal environment designed for a P2 pup may be “too warm” for the larger P12 pup. The ventilatory response to CO₂ is influenced by environmental and body temperature and the effects are age dependent with the greatest effects in the youngest pups (Malcolm et al. 1996; Saiki et al. 1996; Cummings et al. 2009b). Thus the age dependent effects of cooling or warming on the ventilatory response to CO₂ itself and/or the changing thermoneutral range with growth could result in apparent trends in the measured response with advancing age.

3.5.3 The importance of sleep cycling in respiratory measurements in immature rodents—Sleep cycling also changes over the first few days of development and is entrained to feeding cycles and the circadian rhythm of the dam. In the youngest animals, sleep predominates, and of the sleep time, most is in a state with many features of REM or AS with frequent twitching and hypotonia. This state can easily be confused with wakefulness. Since eye opening does not occur until ~ P12, this cannot be used as a sign of wakefulness in the very young pups. In contrast, quiet immobility is a reasonable sign of QS. However, even with nuchal EMG measurements, this behavioral state cannot be completely differentiated from “non-phasic” REM sleep (Seelke et al. 2008). During development there is a decrease in the amount of AS and an increase in the amount of QS and wakefulness. Thus in the very young animal state may change several times over a single 5–10 minute exposure to hypoxia or hypercapnia. Superimposed on this normal cycling is the varying effect of hypoxia or CO₂ on state and arousal. For example in recent experiments, we explored the effect of hypoxia (10% oxygen) on arousal, where the onset of hypoxia was always during quiet immobility. P5 pups switched to REM before arousal nearly 100% of the time, P15 pups 50% of the time, and P25 animals 2% of the time. Moreover, with both hypoxia and hypercapnia, the time to arousal exhibits a kind of “habituation” with progressively increases in latency with repeated exposure. Figure 8 shows the time to arousal (latency) to a brief exposure to either 10% oxygen/90% nitrogen or 8% CO₂/21% oxygen in P15 pups at thermoneutrality. Note that hypoxia is a more potent stimulus for arousal and that both hypoxia and hypercapnia result in arousal “habituation”, a progressive blunting of arousal with repeated exposure. Similar to our previous observations during hypoxia, hypercapnia resulted in a switch from QS to REM sleep in 50% of the experiments in P15 pups (Darnall, RA, unpublished observations).

3.5.4 The importance of the relationship of time after feeding to respiratory measurements—Another factor that is rarely taken into consideration is the time between

feeding and exposure to CO₂. Increases in metabolic rate and decreases in activity associated with feeding might also variably affect $\dot{V}_E$ measurements. The time before or after feeding is routinely taken into consideration in human studies where it has been suggested that the human infant is rarely in a steady state but is either “recovering from the last feed or seeking the next one”. In newborn rat studies, some investigators have eliminated this confounder by feeding the pups by gavage before each experiment (Blumberg et al. 2005).

3.5.1 Sites of central chemoreception in the newborn rat—There is limited information about the sites or developing function of central chemoreception in the developing newborn rat. Single unit recordings in the neonatal brainstem-spinal cord preparation obtained from a region between the caudal end of the vagal roots and 0.5 mm rostral to the caudal end of the hypoglossal roots and from the ventral surface to a depth of 500 μ m showed variable responses to hypercapnia; most of the recorded neurons were not active in phase with respiration (Okada et al. 1993). More recently, neuronal recordings from at least four regions thought to be involved in central chemoreception including the MR, the LC, the NTS, and the pfRG/RTN region in slices and reduced neonatal brainstem-spinal cord preparations and have provided some clues (Putnam et al. 2005; Onimaru et al. 2008). LC neuronal activity measured in brain slices using perforated patch techniques increased by ~44% in response to hypercapnic acidosis at all ages from P1-P21 tested (Stunden et al. 2001). Similarly, hypercapnia activated about 40–50% of NTS neurons in slices from several ages between P1 and adult and the firing rate increase with CO₂ was constant across ages (Conrad et al. 2009). In contrast to neurons isolated from neonatal NTS or the LC, neurons in slices or culture isolated from the MR appear to be only minimally responsive to CO₂ before P12 but thereafter exhibit increasing chemosensitivity (Wang et al. 1999). Thus the increase in neuronal activity in response to CO₂ parallels the increase in the ventilatory response to CO₂ that occurs starting around P12.

The pfRG contains a group of neurons with respiratory related bursts of activity that were originally identified just ventral to the caudal edge of the facial nucleus in the neonatal brainstem-spinal cord preparation (Onimaru et al. 2006). This group of neurons, which has been identified as early as E19 in the fetal rat and which has intrinsic bursting properties at birth (Onimaru 1995, 2002), has been postulated to be the fetal and early neonatal precursor to the RTN, a major site of central chemoreception in the adult (Guyenet et al. 2009). The most rostral and superficial pfRG neurons located very close to the ventral medullary surface under the facial motor nucleus have the same phenotype as the RTN neurons in the adult. They express Phox2b and possibly VGLUT2 and are not catecholaminergic (Guyenet et al. 2009). Moreover, they are postsynaptically sensitive to CO₂ (Onimaru et al. 2008). The intrinsic bursting property present at birth disappears after P7 suggesting that this group of neurons may be particularly important for maintaining breathing at the time of birth. This is supported by convincing evidence showing that severe hypoventilation in neonates occurs in two genetic models of the Congenital Central Hypoventilation Syndrome (CCHS) in which the pfRG is absent but more caudal regions, including the pre-Bötzinger complex, continue to be present (Dubreuil, 2008; Pagliardini, 2008).

4. Conclusions

In conclusion, central chemoreception is active early in development and likely drives fetal breathing movements, which are essential for maintaining fetal lung volume and lung growth and development and in preparation for continuous breathing required after birth. In the fetus breathing is influenced by the combination of behavioral state and powerful inhibition from sources including prostaglandins, adenosine, and neurons located in the upper lateral pons. Inhibition increases during hypoxia and decreases during cooling, cord

compression, in response to increasing PaCO_2 and after the fetus is separated from the placenta at birth. The ventilation of the premature human infant and the neonatal rat responds to CO_2 at birth. In the human the steady state ventilatory response to CO_2 increases with development. In the newborn rat, sensitivity of the CO_2 ventilatory response is either relatively flat or decreases until about P8-10 and increases thereafter. The premature human infant is perhaps more vulnerable to respiratory instability than the newborn rat and exhibits periodic breathing that is augmented by hypoxia and eliminated by breathing oxygen or CO_2 or the administration of respiratory stimulants, including caffeine. Most apnea in premature infants is associated with periodic breathing and pharmacological respiratory stimulants such as caffeine are used clinically. The exact sites of central chemoreception active in the fetus are not known, but may involve the parafacial respiratory group, a likely precursor to the RTN, which contains non-catecholaminergic neurons expressing Phox2b and VGLUT2 that are thought to be a major site of central chemoreception in the adult. Serotonergic neurons located in the midline raphe and noradrenergic neurons in the LC may play important state related roles in the young neonate and adult by enhancing CO_2 sensitivity. The brainstem spinal-cord neonatal and fetal rat preparations promise to provide increasing information about respiratory control and central chemoreception in the developing rodent. Information gleaned from rodent experiments both in the fetus and neonate will have an important impact on the clinical care of human unborn fetuses and premature infants and provide insights into the etiology of important clinical problems, including The Sudden Infant Death Syndrome, Congenital Central Hypoventilation Syndrome, and periodic breathing and apnea of prematurity.

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Figure 1.

Relationship between state related fetal breathing and level of tonic inhibition. The line alternating between HV ECoG and LV ECoG represents alternating combinations of excitation and inhibition that increase the probability of breathing during LV-ECoG and decrease the probability of breathing during HV-ECoG. The shaded box indicates a gradient of tonic inhibition from little or no inhibition (lighter) to heavy inhibition (darker). The boxes in the right side of the diagram indicate known sources of respiratory inhibition and excitation in the fetus. The three dashed lines represent examples of 1) a baseline level of inhibition that results in breathing only during LV-CoG, 2) heavy inhibition as might be encountered during hypoxia where there is no breathing in either state, and 3) little or no inhibition as might occur during cooling or at birth where breathing becomes continuous, occurring during both states.

Figure 2.

The relationship between the percent of active sleep (AS) (short dashed line) and percent of irregular breathing (heavy dashed line) from 30 to 40 weeks postmenstrual age (PMA). Note that the percentage of quiet sleep (QS) steadily increases and the level of indeterminant sleep (IS) steadily decreases from 30 to 40 weeks PMA. The apparent increase in the percentage of AS that occurs from 30 to 36 weeks may be partially because AS cannot be scored with certainty and any sleep that is clearly not AS or QS is scored as IS. The sleep data is from Mirmiran, et al ((Mirmiran et al. 2003), and the irregular breathing data was taken from Parmelee, et al (Parmelee et al. 1972).

Figure 3.

Periodic cycle duration is inversely related to postconceptional age. Periodic cycle duration is defined during periodic breathing as the interval from the beginning of one cluster of breaths to the beginning of the next cluster. The filled circles are data from full term infants and the open circles are from premature infants. Note that the developmentally younger infants, whether full term or premature have longer cycle durations. From Wilkinson, et al (Wilkinson et al. 2007).

Figure 4.

The relationship between the proximity between eupneic PaCO_2 and apneic threshold, oscillations in PaCO_2 , and apnea in neonates and adults. The upper panel represents the neonate where the eupneic PaCO_2 is close to apneic threshold in contrast with the adult, shown in the lower panel where there is a greater difference between PaCO_2 and apneic threshold. Note that similar levels of oscillation of PaCO_2 results in frequent decreases below apneic threshold in the neonate resulting in the apnea associated with periodic breathing. From Rigatto (Rigatto 2003).

Figure 5.

Periodic breathing in the *Mecp2*^{+/-} Mouse during hypoxia, normoxia and hyperoxia. The experiment was performed by exposing the animal to 30 min of 40% oxygen, 12% oxygen, or room air. The upper panel illustrates a mostly regular breathing pattern during hypoxia. The middle and lower panels show periodicity associated with both normoxia and hyperoxia, respectively. From Bissonnette and Knopp (Bissonnette et al. 2008).

Figure 6.

Change in the ventilatory response to CO₂ with advancing age in the rat: a comparison of two methods. In one set of experiments (Stunden et al. 2001), the slope of the $\Delta\dot{V}/\Delta\%CO_2$, normalized to body weight, for each animal was calculated using several levels of CO₂ at different ages. Each grey symbol represents the slope from a single animal. In another set of experiments (Davis et al. 2006) the CO₂ response was calculated as the % of eucapnic $\dot{V}_E$ averaged from several breaths between 3–5 minutes of CO₂ exposure. The open circles represent averages of measurements on several animals at different ages. Lines have been fitted to the data to demonstrate the trends. Values for the slope data is shown on the left axes and values for the % eucapnia data is shown on the right axis. The ranges have been aligned to approximate the two groups of data. Adapted from Stunden et al (Stunden et al. 2001) and Davis et al (Davis et al. 2006).

Figure 7.

The effect of facial cooling on metabolic rate in a premature infant when abdominal temperature is kept constant. Infants were studied on a radiant warmer and facial cooling was accomplished by shading the face from the radiant heat. Note the steady increase in oxygen consumption with decreasing cheek temperature. Darnall, RA, 1978, unpublished observations.

Figure 8.

Arousal habituation in response to repeated exposures either to 10% oxygen or 8% CO₂ in the P15 rat pup. Each trial consisted of 3 minutes of either oxygen or CO₂ followed by 6 minutes of room air. Each trial was started during a period of quiet immobility, most likely QS. Filled triangles represent data from hypoxia experiments and open triangles represent data from hypercapnia experiments. Note the progressive increase in the time to arousal (latency) for both. All studies were approved by the Dartmouth College Institutional Animal Care and Use Committee. Darnall, RA, unpublished observations.