Mechanisms regulating hypoxic respiratory depression during fetal and postnatal life

JOHN M. BISSONNETTE
Departments of Obstetrics and Gynecology and Cell and Developmental Biology, Oregon Health Sciences University, Portland, Oregon 97201–3098

Bissonnette, John M. Mechanisms regulating hypoxic respiratory depression during fetal and postnatal life. Am J Physiol Regulatory Integrative Comp Physiol 278: R1391–R1400, 2000.—Selected topics in the respiratory response to acute hypoxia in the fetus and newborn are reviewed. Peripheral chemoreceptors acting through ionotrophic glutamate receptors play an important role in affecting the initial augmentation phase. Whether fall off in peripheral chemoreceptor activity contributes to the secondary depressive phase remains controversial. A number of approaches including permanent electrolytic and reversible cooling lesions, Fos protein activation, and double-labeling immunohistochemistry has converged to show that an area in and around the locus ceruleus in the rostral pons affects the central depression. There is evidence that this is mediated by catecholamines acting at \( \alpha_2 \)-adrenergic receptors. Tonic activity in early expiratory (postinspiratory) neurons may contribute to hypoxia-induced apneic episodes in the fetus and newborn. Desensitization of \( \alpha \)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors has been demonstrated in respiratory-related neurons both in vivo and in vitro. The role that this process might play in the depressive phase of the hypoxic ventilatory response has not been established. In vitro experiments with isolated brain stem-spinal cord preparations or transverse brain stem slices usually involve anoxia, whereas whole animal experiments use 8–15% O₂. Therefore, caution must be exercised in attempting to construct a unifying framework from these two approaches.

biphasic ventilatory response; control of respiration; fetal breathing movements

NEARLY FIFTY YEARS AGO Cross and co-workers (24, 25) demonstrated that both term and preterm infants exposed to an acute hypoxic environment show an initial increase in minute ventilation that, after the second minute, declined toward baseline in term and below baseline in preterm neonates. Twenty years later, in unanesthetized fetal sheep, it was observed that acute hypoxia resulted in an immediate decline in breathing movements, usually to apnea (15). The mechanisms underlying this hypoxic respiratory depression in the fetus and newborn have received a large amount of attention in subsequent years and have been the subject of a number of comprehensive reviews (7, 26, 43, 71, 75, 78, 88, 100). The present paper is an attempt to review selected aspects of this topic that have appeared recently and/or received less emphasis in the past. Except where contributions from work in adults address unique aspects, the review is confined to the fetus and newborn. In addition, only the response to short-term (5–15 min) hypoxia is reviewed.

INTEGRATED RESPONSE TO HYPOXIA

Figure 1 is a composite of a number of studies in various species at early postnatal and juvenile stages of development. All show an initial increase in minute ventilation \( (V_e) \) followed usually within 1 or 2 min by a relative decline. These two components of the biphasic response to hypoxia are usually termed the augmentation or first phase and the depressive or second phase, respectively. Two common features, are seen across species: the initial augmentation in \( V_e \) is less, relative to the baseline value, at the earlier postnatal ages, and the magnitude of the later depressive phase (whether expressed relative to baseline or as the difference between the augmentation level and that observed in the depressed phase) declines with advancing age. In
situations in which the level of inspired oxygen has been varied, usually between 0.14 and 0.06, the augmentation phase has been greater at the lower fractional inspired oxygen (FIO2) level (17, 18, 31, 61, 67, 103). The increase in VE during the augmentation phase results from a combination of a rise in tidal volume and respiratory frequency. In general, during the depressive phase, tidal volume falls to a greater extent than frequency, and in some instances, respiratory rate continues near the level reached during augmentation (18, 31, 67). In preterm human infants, however, a decline in respiratory frequency is the major contributor to the depression seen in the later stages of the hypoxic challenge (24, 86). These studies in infants were carried out at an FIO2 of 0.15. In neonatal rats, Eden and Hanson (31) have shown that at this degree of hypoxia, the depressive phase is characterized by a decline in frequency, whereas at lower fractional oxygen concentration, tidal volume plays a larger role. Thus the observations in humans may be related to oxygen concentration. The differences in response to hypoxia during the augmentation and depression phases seen in Fig. 1 are probably not related to species differences. Rather, they most likely represent the relative maturity of the respiratory center at the time of birth. Thus 1- to 5-day-old rats and cats resemble preterm humans, and 7- to 8-day-old monkeys resemble 14- to 15-day-old rats and cats.

In fetal sheep, the majority of studies have used a design in which arterial oxygen saturation is reduced from its baseline value of >60% to levels <30%. Koos and co-workers, using the hourly incidence during which breathing movements were present as the index of respiratory drive, showed no change with saturation reduced to 45% and then a progressive linear decline at saturations of 37 and 28% (59). More detailed analysis at fetal O2 saturation of 33% showed no change in either amplitude or frequency in the residual fetal breathing (94). Rurak and Cooper (93) employed an alternate design in which fetal oxygen tension was raised by having the ewe breathe 50% O2 in N2 and then room air. Making allowances for the time lag for the changes to occur in fetal arterial oxygen tension, the results are qualitatively similar to those seen in the newborn. Whereas there was no change in respiratory rate, amplitude increased for ~2 min and then declined to baseline. Thus under conditions nearer those used for the neonate, the fetal integrated responses to hypoxia are similar. To place the depressive phase in context, a brief discussion of the augmentation phase is indicated.

**AUGMENTATION PHASE**

In newborn sheep (17) and rats (37), carotid body denervation eliminates the augmentation phase of the hypoxic ventilatory response, indicating the prominent role of peripheral chemoreceptors. Activity of carotid chemoreceptors in fetal sheep increases on exposure to hypoxia (13), suggesting that the failure to show an augmentation phase is not due to immaturity of peripheral afferents. The sensitivity of neural responses of the
carotid chemoreceptors to hypoxia increases with postnatal age in cat (20, 65, 102), sheep (13), and term infants (19). This maturation of the response to hypoxia has also been demonstrated in rat carotid body in vitro (53). The mechanisms underlying this resetting of the carotid body chemoreceptors have been reviewed (22, 44, 47). To examine the central processing of carotid body afferents, Lawson and Long (64) compared phrenic neural activity responses to electrical stimulation of the carotid sinus nerve in piglets age 1.5–11 days to those 19 days and older. The phrenic equivalent of VE (peak phrenic neuronal activity × frequency) showed a sustained increase that was not different during the 60-s carotid sinus nerve stimulation in these two age groups. There was, however, a difference in the components of minute output. The younger animals showed a decline in frequency coupled with an increase in peak phrenic activity, whereas the older piglets increased frequency for 10 s, but then it returned to baseline level. This response at both ages is different from the integrated response to hypoxia, in which both frequency and tidal volume increase during the augmentation phase. In adult rat and cat, it has been shown that the caudal hypothalamus modulates the respiratory response to hypoxia (see Refs. 48 and 49 for recent reviews). The excitatory output from this area takes place in the absence of input from the carotid sinus nerve. If similar suprapontine responses to hypoxia are present in the newborn, this may explain the difference between carotid nerve stimulation and the augmentation phase of the integrated hypoxic response.

Studies to determine the central neuronal groups to which carotid afferents project and the neurotransmitters involved in the chemoreceptor reflex have been primarily carried out in adult animals. Erickson and Milhorn (33) used immunohistochemical double labeling to identify rat brain stem neurons, which were activated by hypoxia or electrical stimulation of the carotid sinus nerve. Fos protein expression was induced in catecholaminergic neurons in the ventrolateral medulla oblongata and the dorsal vagal complex as well as the locus ceruleus and A5 cell group in the pons. Activity was seen in serotoninergic neurons in the nucleus raphe pallidus, nucleus raphe magnus, and along the ventral medullary surface. Fos-like immunoreactivity also appeared in a number of regions, including the lateral parabrachial and KöllikerFuse nuclei, but there, cells did not colocalize for either catecholamines or serotonin. Microdialysis has been used to demonstrate, in unanesthetized rats, that hypoxia induces an increase in extracellular glutamate in the caudal nucleus of the solitary tract (NTS) during the increase in VE (69). These studies were extended to show that 1) after carotid body denervation, there was neither glutamate increase nor hyperpnea; 2) NTS glutamate injections increased VE, and the broad-spectrum excitatory amino acid antagonist kynurenate reduced the hypoxic ventilatory response (69); and 3) kynurenate application reduced the activation of NTS neurons induced by carotid sinus nerve stimulation (104). In anesthetized rats, more specific antagonists have shown that both the N-methyl-D-aspartate (NMDA) and non-NMDA subtypes of glutamate ionotropic receptors mediate the increase in phrenic burst amplitude evoked by hypoxia (23). The increase in burst frequency, however, was not changed by application of these antagonists to the phrenic motor nucleus. In contrast, systemic administration of an NMDA antagonist to unanesthetized rats markedly blunted the hypoxic ventilatory response by reducing the frequency rise, whereas tidal volume was unaffected (79). Afferents from the lung also play a role in the pattern of respiratory response during the augmentation phase. In awake sheep (12–18 days of age) with vagotomy, breath frequency was little affected by hypoxia due to an inability to shorten expiratory time (28). Tidal volume in these experiments did increase so that the overall response matched that of intact sheep.

Although carotid chemoreceptor and pulmonary afferents input play an important role in the augmentation phase of the hypoxic ventilatory response, in vitro studies have demonstrated a biphasic pattern in neonatal brain stems. These in vitro studies are generally carried out with anoxia, in which O2 is completely replaced by N2 so that the mechanisms responsible for changes in neuronal function may not be the same as those seen in intact unanesthetized preparations subjected to an FiO2 of 0.08–0.15. In an isolated neonatal rat brain stem preparation, anoxia resulted in a transient resting membrane potential depolarization of inspiratory neurons, which was followed by a hyperpolarization (88). Mironov and Richter (68) have extended these observations in 4- to 12-day-old mice using medullary slice preparations to show that hypoxia induced glutamate release, acting at postsynaptic metabotropic receptors, activates L-type Ca2+ channels. Blocking L-type Ca2+ channels eliminated the augmentation phase of the hypoxic response. The in vitro preparations have also been shown to have a developmental pattern in the response to hypoxia. In transverse brain stem slices, which contain the hypoglossal nerve rootlets, hypoxia results in an initial increase in the frequency of rhythmic bursts for early neonatal mice (postnatal day 0–7 (P0–P7)), whereas older mice (P8–P22) demonstrated an increase in both frequency and amplitude (84). Thus the in vitro preparation shows some of the developmental changes observed in intact preparations (see Fig. 1 in which VE in rat is greater at P7–P10 compared with P3–P5). In this same transverse brain stem slice preparation, whole cell patch recordings from inspiratory neurons (defined by discharging in phase with hypoglossal rootlet activity) have shown that during augmentation, the amplitude of synaptic drive potentials increased in slices from mice older than P8. In contrast, slices from P0–P4 mice showed no change in drive potential during anoxia (85).

DEPRESSION PHASE

A number of mechanisms have been put forward to explain the secondary or depression phase of the hypoxic ventilatory response in the newborn, some of which apply to the hypoxic respiratory depression seen
in the fetus. These include a time-dependent decrease in carotid body stimulation, a time-dependent increase in cerebral blood flow with central carbon dioxide washout, a time-dependent decline in metabolic rate and therefore CO₂ production, changes in pulmonary mechanics, and depression in respiratory-related central neurons affected either or both by neurotransmitters or neuromodulators or by membrane properties of the neurons involved (43, 71–73, 75, 88, 101). In this section, emphasis is placed on the role of the peripheral chemoreceptors, the site(s) of central neuronal inhibition, the reciprocal inhibition by early expiratory (postinspiratory) neurons, and the role of catecholamines, and of adenosine.

PERIPHERAL CHEMORECEPTORS

The contribution of the peripheral chemoreceptors to the depressive phase of the hypoxic ventilatory response has been considered from two aspects. 1) Does the afferent output from the carotid body in particular decline over a similar time span as the drop off in Vₑ, and 2) are carotid body afferents necessary to evoke the depression of central neurons.

Different results have been reported in experiments examining the temporal response of the carotid sinus nerve to hypoxia. In pentobarbital sodium anesthetized cats, both Marchal et al. (65) and Carroll et al. (20) showed a time-dependent decline in chemoreceptor function in animals <10 days of age. The timing of the decline was somewhat shorter than that observed to reach the peak increase in minute volume in unanesthetized cats of the same age (67) from a separate study. In contrast, Blanco et al. (14), also using pentobarbital sodium, found that carotid chemoreceptor discharge reached a peak at the same time as the peak rise in Vₑ and remained elevated, whereas minute volume decreased primarily by a decrease in respiratory frequency. Studies in piglets between P1 and P20 support a role for declining carotid afferents in the depression phase. The unsustained response to hypoxia was found more often in younger piglets (74) and occurred over a similar time course as the decline in Vₑ shown in this species in a separate study (63). Carroll and Bureau (21) used breaths of 100% O₂ to assess chemoreceptor function during hypoxia (F₁O₂ = 0.08) and found that in 2- to 3-day-old lambs, it was reduced after 15 min of hypoxia but not at 7 min. Older lambs (10–11 days) showed no decline in the response to hyperoxia.

Fetal sheep studied with carotid denervation and vagotomy became apneic with hypoxia (60) similar to intact animals. The onset of the apnea, however, was delayed compared with fetuses with intact chemoreceptors, suggesting that these afferents play a role in the respiratory depression. In chemodenervated 4-day-old lambs, there was little depression when breathing 0.07 O₂, again supporting a role of the carotid body (17). In neonatal rats, however, a depression phase characterized by declines in both peak integrated phrenic activity and respiratory frequency was seen in animals with bilateral sectioned carotid sinus nerves (37). Thus whether carotid body input is essential for the expression of the depressive phase has not been unequivocally established.

SITE(S) THAT MEDIATE CENTRAL RESPIRATORY DEPRESSION

In 1983, Dawes and co-workers demonstrated that transverse section of fetal sheep brain stem at the upper pons or midcollicular altered the response to hypoxia (27). In place of the expected respiratory depression, often to apnea, these transected fetuses showed an increase in both respiratory frequency and breath amplitude measured from a tracheal pressure catheter. This hyperpnea was maintained for the 10-min hypoxic challenge. Martin-Body and Johnson (66), in unanesthetized (5–10 day old) rabbits, showed that this result was not confined to the fetus. Transected (at the midbrain–pons junction) animals showed a maintained stimulation of Vₑ during 15 min of exposure to 7% oxygen, which was characterized by an increase in both frequency and tidal volume. Gluckman and Johnson (41) used electrolytic lesions in fetal sheep to localize the site that mediates the hypoxic depression to the region of the upper lateral pons at, and slightly rostral to, the sensory and motor nuclei of the trigeminal nerve. Walker and associates have further established the importance of the dorsolateral pons by examining Fos immunoreactivity as an index of neuronal activity in hypoxic fetal and newborn (7–18 day old) sheep. An area just ventral to the medial parabrachial nucleus near the Kölliker-Fuse nucleus, which they have termed the subceruleus nucleus (Fig. 2) was shown to be unique in that hypoxia induced Fos immunoreactivity in fetal but not newborn sheep (16). This suggests that this area may be important for the more profound hypoxia-induced respiratory depression in the fetus. Interestingly, in newborns with carotid body denervation, hypoxia resulted in Fos immunoreactivity in the subceruleus consistent with the proposal that in neonates, peripheral afferents inhibit neuronal activity in this area. The ventilatory response, however, is not supportive in that chemodenervated newborn sheep show little depression to hypoxia (17). The interpretation is complex in that these animals also fail to have a significant augmentation. Nitsos and Walker (76) have further defined the function of the subceruleus neurons that are Fos immunoreactive by demonstrating that 1) they are catecholaminergic, but not cholinergic or GABAergic, and 2) cholera toxin B conjugated to colloidal gold particles demonstrated that a proportion of them project to the C5–C8 ventral horn. In 3- to 8-day-old sheep, cooling in the area of the locus ceruleus prevented hypoxic depression (70). In an exacting experimental protocol, Koos et al. (55) transected the brain stem (at varied locations between the rostral midbrain and the pontomedullary junction), and, after these fetal sheep had been shown to increase the rate and amplitude of breathing during hypoxia, they were chemodenervated at a second surgical procedure. Hypoxia no longer stimulated breathing after removal of carotid and aortic afferents. These experiments add considerably to the conclusion that the fetus has intact...
peripheral chemoreceptors whose excitatory activity during hypoxia is suppressed by central mechanisms. These results were confirmed by Johnston and Gluckman (50) using a similar two-stage protocol, in which electrolytic lesions in the rostral lateral pons were followed by chemodenervation. It should be pointed out that these brain stem transection and rostral lateral pontine lesion preparations in fetal sheep disrupt the normal respiratory pattern. Under basal conditions, the lesioned fetuses have a respiratory frequency of 15–20 per minute, less than one-third that of intact sheep. Hypoxia increases frequency to 30–40 breaths per minute, a value still below the eupneic level of intact animals (27, 41, 50, 55). This alteration in eupneic respiratory rate was not seen in unanesthetized neonatal rabbits transected at the midbrain-pontine junction (66).

Whereas considerable emphasis has been placed on the rostral lateral pons, a number of areas rostral to the pons has also been implicated in affecting hypoxic respiratory depression. Building on the work of Gallman and co-workers (39) in adult cats, Waites et al. (99) made electrolytic lesions in the red nucleus of decerebrate rabbits (28–35 days old) and observed that bilateral (but not unilateral) lesions abolished the depressive phase of the hypoxic ventilatory response. These studies were extended to show that neuronal stimulation with nanoliter volumes of glutamate interrupted rhythmic phrenic discharge (Fig. 3) (1). In addition, with the use of midbrain slices, it was shown from extracellular recordings that neurons within the red nucleus increase their firing rate when O2 is reduced from 95% to 45% in the artificial cerebrospinal fluid (CSF) bathing the slice (1). In fetal sheep, hypoxia

![Fig. 2. Hypoxia induced Fos immunoreactivity in fetal and newborn sheep.](image1)

![Fig. 3. Effect of glutamate injection within red nucleus on phrenic neurogram in neonatal rabbit.](image2)
did not cause Fos immunoreactivity in the red nucleus, although some was seen ventral to it (77). More recently, ibotenic acid has been used to lesion neuronal populations in the diencephalon of fetal sheep (56). This approach has the advantage of leaving fibers of passage and vascular supply more intact than with thermal destruction. The parafascicular nuclear complex in the thalamus was identified as a site mediating the hypoxic ventilatory depression in sheep fetuses (56). In contrast to brain stem-transected and rostral lateral pons lesioned sheep fetuses, these thalamic lesioned animals had respiratory frequencies nearer to intact animals [47 ± 12 breaths/min vs. 70 ± 7 (SE)], but hypoxia insignificantly reduced frequency and amplitude compared with the increases seen in the more caudal lesions or transections (see above). As with the red nucleus, no increase in Fos immunoreactivity was observed after hypoxia in this parafascicular nucleus region (77). These results indicate that, in contrast to the dorsal lateral pontine sites, the thalamic region does not receive excitatory input from peripheral chemoreceptors during hypoxia.

NEUROTRANSMITTERS AND NEUROMODULATORS MEDIATING THE DEPRESSION PHASE

A number of both classical neurotransmitters and neuromodulators have been put forward as important mediators of hypoxic respiratory depression in the fetus and newborn. Opioids, nitric oxide, and substance P show evidence of their roles and have been reviewed along with other effectors (7, 75, 88, 95). In this section, the involvement of catecholamines acting at α2-receptors and adenosine at A1 receptors will be considered. The demonstration that rostral lateral pontine (subce- ruleus) neurons implicated in respiratory depression in the fetus are catecholaminergic (see above) underscores the interest in this neurotransmitter. Systemic infusions of the α2-adrenergic-receptor agonist clonidine inhibited the incidence of breathing in fetal sheep (3), and this was blocked by the antagonist idozoxan. The site of action for clonidine was further localized by administering clonidine into a lateral cerebral ventricle. These infusions reduced the incidence of breathing by 66%, and the duration of breathing episodes was less than one-half that seen in the control period (4). Systemic and lateral ventricular infusions of α2-adrenergic antagonists did not affect fetal breathing, indicating that these receptors are not tonically active under eupneic conditions (2, 3). The importance of α2-adrenergic-receptor activation during hypoxia in the fetus was studied during antagonist infusions into the lateral cerebral ventricles. These experiments showed that in contrast to infusions of CSF, fetal breathing was maintained in the 30-min hypoxic challenge (2). Similar to brain-transected or pontine lesioned fetuses, breath amplitude was significantly augmented during hypoxia. These studies in fetal sheep have been confirmed with systemic infusions of a combined α1- and α2-adrenergic-receptor antagonist that prevented hypoxic respiratory depression (40). In vitro newborn rat brain stem preparations, in which solutions bathing the pons can be isolated from those bathing the medulla, have shown that norepinephrine increases cervical root burst frequency when it acts at the pontine level (34). In contrast, norepinephrine slows burst frequency when it is applied to the medulla, and α2-adrenergic-receptor antagonists blocked this depression. Studies in brain stem slice preparations from 18- to 32-day-old rats revealed that α2-adrenergic agonists produce hyperpolarization in hypoglossal motoneurons by decreasing the amplitude of a hyperpolarization-activated inward current (80). Hypoxia has been shown to result in membrane hyperpolarization of most respiratory neurons in the ventral respiratory group of neonatal rat isolated brain stem preparations (88). In slice preparations from neonatal mice, however, depolarization is seen in only a minority of inspiratory neurons (85). In the brain stem preparation experiments (88), removal of chloride from the whole cell patch pipette did not affect the change in membrane potential, suggesting that hypoxia was activating potassium channels. Activation of α2-adrenergic receptors stimulates K+ currents (96), which indicates that their role in hypoxic inhibition may involve multiple cellular mechanisms. Taken together, these observations are consistent with an important function of α2-adrenergic receptors in the depression phase of the hypoxic ventilatory response. Adenosine has been shown in the fetus and newborn to fulfill a number of the criteria used to establish a significant role for a neuromodulator in physiological functions. Adenosine A1 receptors are present in respiratory related regions of the brain stem of fetal sheep (12). During the moderate hypoxia, which results in an inhibition of the incidence of fetal breathing, microdialysis from probes placed in the midbrain showed a 2.3-fold increase in perfusate adenosine concentration (57). Adenosine or its analogs administered either systemically or into the fourth cerebral cerebral ventricle causes respiratory depression in newborns and fetuses (9, 58, 62), which can be overcome with adenosine-receptor blockade. In addition, the hypoxia-induced depression can be abolished or attenuated with these xanthine derivatives in both intact fetus (8) and newborn (32, 92) and in isolated neonatal brainstem spinal cord preparations (52). Adenosine can inhibit neuronal activity by pre- and postsynaptic mechanisms (97). Recordings from hypoglossal motoneurons in neonatal rat brain stem slices demonstrated that adenosine receptor agonists decreased the amplitude of glutamate-evoked excitatory postsynaptic potentials (EPSPs) (5), indicating a presynaptic mode of action. In these same studies, adenosine did not change hypoglossal neuronal input resistance or membrane potential, consistent with a lack of postsynaptic activity. Similar approaches were used to examine the effect of adenosine on phrenic motoneurons in an in vitro isolated brain stem-spinal cord preparation (29). Adenosine analogs significantly decreased the frequency of spontaneous and of miniature EPSPs. These compounds, however, had no effect on phrenic input resistance, and the inward currents produced by exogenous glutamate also were unaffected.
indicating a lack of postsynaptic effects. More recently, adenosine neuromodulation has been studied in vitro in the rostral ventrolateral medulla of neonatal rats (46). The type of respiratory-related neuron was identified by the temporal relationship of its membrane trajectory with activity in the cervical four (C4) rootlet. Adenosine resulted in a slowing of burst rate from C4 and a shortening of discharge duration in inspiratory neurons. Whole cell recordings of inspiratory neurons revealed that neither membrane potential nor input resistance were changed by adenosine; however, spontaneous postsynaptic activities were decreased, indicating a presynaptic mode of action. In contrast, expiratory neurons responded to adenosine with a hyperpolarization of their membrane potential and a reduction in input resistance. These membrane property changes persisted in the presence of tetrodotoxin to block synaptic activity consistent with postsynaptic action of adenosine on expiratory neurons (46). Interestingly, C4 discharge continued, albeit at a slower rate, despite expiratory neuron silencing (see RECIPROCAL INHIBITION IN THE RESPIRATORY NETWORK). In juvenile rats, recordings from CA1 neurons of the hippocampus demonstrated that an adenosine A1 antagonist prevented the hypoxia-induced depression of excitatory postsynaptic potentials (51). Adenosine-receptor blockade during hypoxia did not, however, eliminate hypoxia’s depression of inhibitory potentials. In addition, synaptic inhibition during the inspiratory phase persists in inspiratory neurons in the presence of adenosine (46). Therefore, this neuromodulator does not account for all the cellular changes seen with hypoxia.

RECIPROCAL INHIBITION IN THE RESPIRATORY NETWORK

Models of the respiratory network include reciprocal inhibition, in which the onset of activity in postsynaptic (or early expiratory) neurons is associated with inhibition of inspiratory neurons (6, 30, 35, 36, 87). In this framework, it can be hypothesized that apnea seen in the fetus and newborn is triggered or maintained by tonic activity in postsynaptic neurons, leading to a long-lasting inhibition of inspiration. Support for this suggestion is seen in a record from an expiratory neuron in an isolated neonatal rat brain stem preparation. The neuron was hyperpolarized by the electrode before hypoxia. During the 4-min period of anoxia, the membrane potential gradually depolarized and a prolonged train of action potentials was seen (Fig. 5 in Ref. 88). Recording from pre-Botzinger area neurons, which resemble in vivo postsynaptic neurons, Ramirez et al. (85) found, at P0–P4, that there was hyperpolarization during the expiratory phase. The hyperpolarization usually seen during hypoglossal bursts in these neurons was lost in the depressive phase of the hypoxic response and replaced by a depolarization, indicating relative loss of inhibition. In older mice, in which apnea developed during the depression phase, expiratory neurons, however, are hyperpolarized and inactive (85). The loss of inhibition, seen in expiratory neurons of younger mice, supports the suggestion made in fetal sheep that hypoxia results in tonic activity of expiratory neurons.

Recordings from expiratory neurons during hypoxia have not been made in fetal sheep. Simultaneous recordings of electromyograms (EMG) from the diaphragm and thyroarytenoid muscle (TA), however, indicate that the latter may be an index of the activity in postinspiratory neurons (10, 45, 54). In eupnea, when phasic TA EMG activity is seen, it has an onset concordant with the arrest of diaphragmatic EMG and its activity stops well before the next diaphragm burst. Hypoxia, both isocapnic and hypocapnic, has been shown to induce tonic activity in TA of fetal sheep both during the expiratory phases that precede apnea and during the apnea itself (10). In newborn lambs (P0–P3), TA EMG activity is seen in the apneic episodes induced by breathing 100% O2 for 5 breaths after 6 min of 8% hypoxia (82). Unlike in the fetus, this tonic TA activity was only seen in hypocapnic hypoxia. In older lambs (P11–P18), tonic expiratory activity was seen in the TA in the apneic episodes that occurred on returning the animals to room air after a hypoxic episode (81). Thus, whereas they are not as compelling as electrophysiologic recordings from defined postinspiratory neurons, these results using TA muscle activity as an index of central neuronal activity suggest that expiratory neurons may play a role in hypoxic respiratory depression in the fetus and newborn. The differences between the unanesthetized whole animal and in vitro reduced preparations may be related to relative hypoxia compared with anoxia.

DESENSITIZATION IN EXCITATORY AMINO ACID RECEPTORS

As discussed in AUGMENTATION PHASE, glutamate is the principal neurotransmitter mediating the afferent input from peripheral chemoreceptors in the augmentation phase of the hypoxic ventilatory response. The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) subtype of non-NMDA inotropic glutamate receptor studied in single neurons or excised membrane patches has been shown to rapidly desensitize in the presence of ligand (83, 98). AMPA receptors are desensitized by steady-state glutamate concentrations (EC50 = 5–10 µM) much lower than that necessary for activation of the receptor (83, 98). With the use of microdialysis to determine interstitial concentrations in the area of the nucleus ambiguous, Richter et al. (89) have recently measured changes in glutamate during hypoxia in anesthetized adult cats. Samples were determined at 1-min intervals and showed an almost 10-fold rise from baseline levels of 9.5 µM in the first minute. If the kinetics for desensitization in respiratory neurons are similar to those determined in chicken spinal neurons (98) and nucleus magnocellularis (83), then a high proportion of AMPA receptors would be in the desensitized state, leading to a loss of excitability. Cyclothiazide, a drug that blocks AMPA desensitization, has been used to examine this question in medullary slices from 1- to 4-day-old rats and in unanesthetized fetal sheep. Cyclothiazide increased both the
frequency and amplitude of hypoglossal rootlet bursting in slice proportions (38). Voltage-clamp whole cell recordings from hypoglossal motoneurons in these preparations demonstrated that inward current was enhanced in the presence of cyclothiazide. In fetal sheep, instillation of cyclothiazide into the CSF of the fourth ventricle resulted in an increase in both amplitude and frequency of fetal breathing (11). The latter result suggests that under in vivo conditions, the steady-state interstitial concentrations of glutamate render a portion of AMPA receptors in the desensitized state. It is not unreasonable to suggest that hypoxia-induced increases in glutamate would lead to further desensitization of these excitatory receptors.

Whereas ligand-induced desensitization of AMPA receptors may contribute to hypoxic respiratory depression in other areas of the medulla, there is evidence that this process does not have an effect in the NTS. Low-frequency stimulation at 5 Hz for 5 min of the solitary tract in transverse brain stem slices from 3- to 21-day-old rats is characterized by a rapidly developing loss of EPSP amplitude. Excitability in NTs neurons declines to less than one-half the prestimulus level (105). Whereas the NTS neurons in this study were not identified as respiratory, the time course of their depression fits very well with the changes in VE seen in intact preparations. Zhou et al. (105) considered AMPA receptor desensitization as a mechanism for this decrease in EPSP amplitude, but cyclothiazide failed to reverse the process. Because the hypoxic ventilatory response in the fetus and newborn has not been examined in the presence of cyclothiazide, the role of AMPA receptor desensitization at this time remains undetermined.

CONCLUSIONS

The understanding of the mechanisms underlying the hypoxic ventilatory response continues to evolve. There is considerable evidence that central mechanisms, especially in the fetus, overcome the excitatory input from peripheral chemoreceptors, resulting in respiratory depression. A goal for future investigation is to determine whether these central mechanisms result from hypoxic activation of discrete neuronal populations that exert an active inhibition or from loss of reciprocal inhibition leading to tonic activation of expiratory neurons.

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Address for reprint requests and other correspondence: J. M. Bissonnette, Rm. 822B, Medical Research Bldg., L-458, Oregon Health Sciences Univ, Portland, OR 97201-3098 (E-mail: bissonne@ohsu.edu).

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