Postnatal expression of neurotransmitters, receptors, and cytochrome oxidase in the rat pre-Bötzing complex

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Liu, Qiuli, and Margaret T. T. Wong-Riley. Postnatal expression of neurotransmitters, receptors, and cytochrome oxidase in the rat pre-Bötzing complex. J Appl Physiol 92: 923–934, 2002; 10.1152/japplphysiol.00977.2001.—The pre-Bötzing complex (PBC) is postulated as the center of respiratory rhythmogenesis. Previously, we found a reduction or plateau of cytochrome oxidase (CO) activity in the PBC and other respiratory nuclei at postnatal days 3–4, despite a general increase of CO with age, suggesting a period of synaptic readjustment. The present study examined the expression of CO and a number of neurochemicals in the PBC at closer time intervals. At postnatal days 3–4 and, more prominently, at postnatal day 12, expression of CO, glutamate, and N-methyl-D-aspartate receptor subunit 1 was reduced, whereas expression of GABA, GABAA receptor, glycine receptor, and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor subunit 2 was increased. These findings are consistent with our hypothesis that decreased CO activity is associated with an increase in inhibitory drive (mediated by GABA and glycine, their receptors, and possibly blockage of Ca2+ entry by glutamate receptor subunit 2) and a decrease in excitatory drive (mediated by glutamate and its receptors). Our findings point to two critical periods during postnatal development of the rat when their respiratory system may be more vulnerable to respiratory insults.

N-methyl-D-aspartate receptor subunit 1; glutamate receptor subunit 2; GABAA receptor; neurokinin-1 receptor; glutamate

THE MECHANISM OF RESPIRATORY rhythmogenesis is not fully understood, but cumulative evidence suggests that the pre-Bötzing complex (PBC) may be the center or kernel of respiratory rhythm generation (12, 37, 38, 42). The PBC is located at the rostroventrolateral medulla, ventral to the nucleus ambiguous (NA), and can be anatomically defined by the distribution of immunoactive neurokinin-1 receptors (NK1R) (15). Removal of only the PBC in the brain stem eliminated respiratory rhythm generation in neonatal rats (42). All six basic types of respiratory neurons were identified in the PBC (9, 42), and neurons with voltage-dependent pacemaker-like properties were also found there (6, 7, 17, 22, 47). It has been implied that the PBC functions as a central hypoxia chemosensor for respiration (44, 45). The application of agonists and antagonists of some neurotransmitters and neuromodulators to the PBC can induce apparent changes in respiratory rhythm and pattern (8, 21, 34, 41, 43). However, very little is known about postnatal development of neurochemicals in the PBC when the system may be more vulnerable to respiratory distress.

Cytochrome oxidase (CO) is the terminal enzyme of the mitochondrial respiratory chain and is considered to be a reliable marker of neurons’ metabolic capacity and levels of functional activity (51). Previously, we found that the PBC and other respiratory nuclei in the rat exhibited a general increase in CO activity with postnatal development. However, there was a distinct decrease or plateau of CO activity between postnatal days 3 (P3) and 4 (P4), suggesting a period of synaptic readjustment (19). On the basis of our previous findings that CO-rich zones were dominated by excitatory synapses (52), we hypothesized that decreased CO activity is associated with a decrease in excitatory drive [mediated by glutamate (Glu) and its receptors] and/or an increase in inhibitory drive [mediated by GABA and glycine (Gly) and their receptors].

The goal of the present study was to perform an even tighter temporal analysis of the postnatal development of CO activity and determine whether it correlated with the expression of several neurotransmitter and receptor candidates in the PBC. The neurochemicals examined were Glu, N-methyl-D-aspartate (NMDA) receptor subunit 1 (NMDAR1), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit 2 [Glu receptor (GluR) subunit 2 (GluR2)], GABA, GABAA receptor (GABAAR), and Gly receptor (GlyR). Neurokinin-1 receptor (NK1R) was used to identify the PBC (15).

MATERIALS AND METHODS

Tissue preparation. A total of 197 Sprague-Dawley rats from 19 litters were used in accordance with National Institutes of Health and Medical College of Wisconsin regulations. One hundred twenty rats from 12 litters were studied from postnatal day 0 (P0) to postnatal day 21 (P21) for CO histochemistry, Nissl staining, and NK1R immunohistochemistry.
Litter size averaged 8–13 pups, and at every time point, 6 rats from 6 different litters were used. On the basis of our CO findings, 77 rats from 7 litters were processed for neurotransmitter and receptor studies between P2 and P21, with 7 rats from 7 different litters at every time point. At the end of each experiment, rats were deeply anesthetized with ether and perfused through the aorta with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PBS), pH 7.4, with 4% sucrose. The brain stems were removed and postfixed by immersion in the same fixative for 4 h at 4°C. They were then cryoprotected in increasing concentrations of sucrose (10, 20, and 30%) in 0.1 M PBS at 4°C, frozen in dry ice, and stored at −80°C until use.

CO histochemistry. Coronal sections of frozen brain stems were cut at 12-µm thickness with a cryostat. At each time point, seven sets of serial sections were mounted on gelatin-coated slides. The sections were blocked overnight at 4°C with 5% nonfat dry milk-5% normal goat serum-1% Triton X-100 in 0.1 M PBS (pH 7.4). They were then incubated at 4°C for 36–48 h in the primary antibodies diluted at the appropriate concentrations in the same solutions used for blocking. The dilutions were 1:10,000 for NK1R (Sigma), 1:500 for GABA (Sigma), 1:1,000 for NMDAR1 (Chemicon), 1:300 for GluR2 (Chemicon), and 1:400 for GABABR (Chemicon). The sections were incubated in the appropriate secondary antibodies: goat anti-rabbit IgG-horseradish peroxidase (HRP; Bio-Rad Laboratories) for NK1R, GABA, NMDAR1, GlyR, and GluR2; goat anti-mouse IgG-HRP (Bio-Rad Laboratories) for Glu; or goat anti-guinea pig IgG-HRP (Chemicon) for GABABR, at 1:100 dilution in the modified blocking solution (without Triton X-100) for 1 h at room temperature. Immunoreactivity was detected with 0.05% 3,3′-diaminobenzidine-0.004% H2O2 in PBS (pH 7.4) for 5–10 min, and the reaction was stopped with cold PBS (pH 7.4). The sections were then washed with cold 0.1 M PBS (pH 7.4) three times and dehydrated, and coverslips were applied.

Immunohistochemistry. Coronal sections of frozen brain stems were cut at 12-µm thickness with a cryostat. For each time point, seven sets of serial sections were mounted on gelatin-coated slides. The sections were blocked overnight at 4°C with 5% nonfat dry milk-5% normal goat serum-1% Triton X-100 in 0.1 M PBS (pH 7.4). They were then incubated at 4°C for 36–48 h in the primary antibodies diluted at the appropriate concentrations in the same solutions used for blocking. The dilutions were 1:10,000 for NK1R (Sigma), 1:500 for GABA (Sigma), 1:1,000 for NMDAR1 (Chemicon), 1:300 for GluR2 (Chemicon), and 1:400 for GABABR (Chemicon). The sections were incubated in the appropriate secondary antibodies: goat anti-rabbit IgG-horseradish peroxidase (HRP; Bio-Rad Laboratories) for NK1R, GABA, NMDAR1, GlyR, and GluR2; goat anti-mouse IgG-HRP (Bio-Rad Laboratories) for Glu; or goat anti-guinea pig IgG-HRP (Chemicon) for GABABR, at 1:100 dilution in the modified blocking solution (without Triton X-100) for 1 h at room temperature. Immunoreactivity was detected with 0.05% 3,3′-diaminobenzidine-0.004% H2O2 in PBS (pH 7.4) for 5–10 min, and the reaction was stopped with cold PBS (pH 7.4). The sections were then washed with cold 0.1 M PBS (pH 7.4) three times and dehydrated, and coverslips were applied.
Cell area measurement. The long and short axes of neuronal cell bodies in PBC were measured with a reticule and a ×40 objective lens in cresyl violet-stained sections. Between 150 and 200 neurons were measured at each age group. The average diameter was calculated, and the cell areas were estimated.

Quantitative densitometry. Optical densitometric measurements of reaction product of CO and immunohistochemistry were performed with a Zeiss Zonax MPM 03 photometer, a ×25 objective, and a 2-μm-diameter measuring spot. White (tungsten) light was used for illumination, and all lighting conditions were held constant for all measurements. The white matter was used as an internal standard for measurements because of its very low levels of CO activity and immunoreactivity. Thus the white matter was set at zero for each section measured. The optical densitometric value of each neuron in the PBC was an average reading of two to four spots in the cytoplasm. Thirty to 80 neurons in the PBC for each rat and a total of 150–350 neurons for each marker (CO or neurochemicals) at each age were measured. The mean optical density values and standard deviations of each marker at each age were then obtained. Statistical comparisons were made between successive age groups (e.g., CO activity at P0 vs. P1 and P1 vs. P2; neurochemicals and receptors at P2 vs. P3 and P3 vs. P4) by using one-way ANOVA (to control for the type I comparisonwise error rate) and Tukey’s Studentized range test (to control for the type I experimentwise error rate). Significance was set at \( P < 0.01 \) for one-way ANOVA and \( P < 0.05 \) for Tukey’s test.

RESULTS

The PBC is located in the mid portion of the rostral-caudal axis of the ventrolateral medulla, ventral to the NA, and between the rootlets of the hypoglossal nerve and the vagus nerve. The total rostrocaudal extent is 300–480 μm at P0–P21. Neurons in the PBC were multipolar, oval, fusiform, or pyramidal in shape, and their sizes were small (75–200 μm²) at P0–P4, small and medium (200–350 μm²) at P5–P13, and small, medium, and large (>350 μm²) at P14–P21. The caudal two-thirds of the PBC contained a higher density of neurons, and the mean size of neurons was larger than in the rostral one-third, suggesting that there may be two functionally differentiated parts. All the photomicrographs were taken from the caudal two-thirds of the PBC.

Fig. 2. Neurons in the PBC histochemically reacted for cytochrome oxidase (CO) at P2 (A), P4 (B), P7 (C), P12 (D), and P21 (E). Arrows, neurons with high (D), moderate (M), and low (L) CO activity. CO activity is generally low at P4 and P12. Scale bars: 20 μm.
NK1R-immunoreactive neurons in the PBC. In the ventrolateral medulla of rats, NK1R-immunoreactive (ir) neurons can be divided into two groups: PBC ventrally and NA dorsally (Fig. 1). NK1R immunoreactivity was expressed mainly along the plasma membrane of neuronal cell bodies and processes that were distributed within the ventral reticular formation. Occasionally, neuronal cytoplasm was also lightly labeled. About 30% of neurons in the PBC expressed NK1R immunoreactivity, and they were multipolar, oval, or fusiform in shape and mainly small or medium in size. A few large neurons were present in the older rats (P14–P21). Long and thin NK1R-ir processes that originated from the PBC usually traversed within the nucleus, toward the middle of the medulla, dorsally to the NA, or ventrally to the surface of the medulla. NK1R immunoreactivity in the PBC was expressed at a moderate level during the neonatal period, with fewer and shorter processes earlier and longer and more profuse processes with increasing age up to P21, the oldest age group examined in this study. Immunoreactivity increased steadily in cell bodies and processes with age, except for distinct reductions at P3–P4 and P12 (Fig. 1).

CO-reactive neurons in the PBC. In the ventrolateral medulla of rats, CO-reactive neurons can be differentiated between those in the PBC (ventrally) and those in the NA (dorsally). They were multipolar, oval, fusiform, or pyramidal in shape and mainly small to medium in size. CO-reactive neurons in the PBC exhibited dark, moderate, or light intensities of CO labeling, with processes traceable for short distances in different directions. Neuronal CO activity generally increased with age, expressing light-to-moderate labeling at P0–P5, and light-to-dark labeling after P5. The percentage of darkly reactive neurons and the level of CO activity in the neuropil increased with age. However, all three metabolic cell types showed a plateau of CO activity at P3, and the darkly reactive neurons remained at this plateau at P4. In addition, all neuronal types exhibited a sharp reduction in CO activity at P12 (Fig. 2, see Fig. 9A).

Neuronal area measurements. The somal sizes of PBC neurons at each developmental age are shown

Fig. 3. Glutamate (Glu)-ir neurons in the PBC at P2 (A), P4 (B), P7 (C), P12 (D), and P21 (E). Glu immunoreactivity is lower at P4 and P12 than at other times. Scale bars: 20 μm.
qualitatively in Figs. 1–8 and quantitatively in Fig. 9B. There was no pause in the continued increase in neuronal areas with age, and near-mature levels were reached at P15 (see Fig. 9B).

**Glu-ir neurons in the PBC.** Glu immunoreactivity was observed in 50% of neurons in the PBC at P0–P14 and in 73% of neurons at P21. Glu-ir neurons were mainly multipolar and oval in shape, with a few fusiform neurons. Glu immunoreaction product was not evenly distributed in the cytoplasm, and Glu-ir processes were not distinct. The postnatal developmental pattern of Glu-ir neurons was similar to that of CO activity. There was a general increase in immunoreactivity with age but a significant decrease at P4 and a more dramatic reduction at P12 (Fig. 3, see Fig. 9C).

**NMDAR1-ir neurons in the PBC.** NMDAR1 immunoreactivity was expressed in 50–80% of neurons in the PBC at P0–P21, but the density of detectable NMDAR1-ir neurons dropped to only 36% at P12. These neurons were evenly distributed throughout the PBC, and they were multipolar, fusiform, oval, or pyramidal in shape and mainly small to medium in size. Their processes were detectable for only short distances. Their postnatal developmental pattern was similar to that of Glu-ir neurons. There was a general increase in immunoreactivity with age but a significant decrease at P4 and a more prominent reduction at P12 (Fig. 4, see Fig. 9D).

**GluR2-ir neurons in the PBC.** GluR2 immunoreactivity was demonstrated in 30–55% of neurons in the PBC. GluR2-ir neurons were mainly multipolar and pyramidal in shape and mainly small to medium in size. Their immunoreactivity was quite high at P2–P4 but slowly declined to a moderate level at P5–P11. At P12, it increased strikingly to a peak level and then slowly decreased to a moderate level at P21 (Fig. 5; see Fig. 9E).

**GABA-ir neurons in the PBC.** Only 15%-30% of neurons in the PBC exhibited GABA immunoreactivity. They were mainly small in size and multipolar, oval, or fusiform in shape. At younger ages (P2–P5), neuronal processes could be more easily traced within the same plane of section than after P5, but the immunoreactiv-
ity of the neuropil increased gradually with age. The postnatal developmental pattern of neurons with GABA immunoreactivity demonstrated two peaks: a smaller peak at P4 and a much more prominent peak at P12 (Fig. 6, see Fig. 9F).

GABA-BR-ir neurons in the PBC. About 30–50% of neurons in the PBC expressed GABA-BR immunoreactivity. The reaction product was mainly granular in shape. GABA-BR-ir neurons were mainly multipolar or pyramidal in shape and small to medium in size. Their immunoreactivity also exhibited two peaks during postnatal development, at P4 and P12, with much higher immunoreactivity at P12 than at P4. At P21, PBC neurons showed a moderate level of immunoreactivity for GABA-BR (Fig. 7, see Fig. 9G).

GlyR-ir neurons in the PBC. About 30–50% of neurons in the PBC showed GlyR immunoreactivity. They were mainly multipolar or oval in shape and small to medium in size. Some large GlyR-ir neurons were present after P14. Their rather long processes extended dorsally, ventrally, and transversely, especially at P4 and P12. Immunoreactivity showed two peaks during postnatal development: a peak at P4 and a much more distinct peak at P12 (Fig. 8, see Fig. 9H).

**DISCUSSION**

CO is a critical oxidative enzyme in the generation of ATP for fueling a variety of neuronal functions. It is a reliable marker of the metabolic capacity and functional activity of neurons (51). Heightened CO activity in the central nervous system (CNS) correlates directly with a greater proportion of excitatory synapses, whereas a predominance of inhibitory inputs is associated with a lower level of CO (23, 27, 28, 51). The reason is that the bulk of energy consumed by neurons is used for repolarizing membranes after excitatory depolarization, whereas repolarization after inhibitory hyperpolarization is mainly passive (51). CO activity in neurons is also correlated with their sustained firing rate generated synaptically or spontaneously (52).

CO activity during CNS development exhibits heterogeneity. In the whole rat brain, most of the increases in CO activity occur at P5–P25 (5). It is low in many brain regions from P0 to P10 and rises between...
P10 and P14, with greater increases between P21 and P35 (2). In the fiber tracts, peak CO activity shifts as a function of postnatal age in a caudal-to-rostral direction. It is maximal at P7 in the spinal trigeminal tract but at P17 in the hippocampal commissure (25). Significant maturation of the ventilatory control system in the rat occurs between P10 and P15 (49).

The present study confirms our previous report of a distinct plateau of CO activity at P3 in the PBC (19). Additionally, it shows a prominent decline in CO activity at P12. At these two ages, and especially at P12, reduced CO activity suggests a transient adjustment in synaptic activity of the PBC, possibly involving decreased excitatory drive and/or enhanced inhibitory drive. The PBC, being the presumed center of respiratory rhythm generation (42) and a central hypoxia chemosensor for respiration (44), may be less responsive to abnormal respiratory demands during these periods of low CO. Consequently, the system may be more vulnerable to failure when stressed by insults such as ischemia, hypoxia, and other respiratory distress. Changes in CO activity and neurochemical immunoreactivity at P3–P4 and P12 occur despite a steady increase in neuronal size with age, indicating that they are not related to cell growth per se. These two periods may be considered “critical periods” of development for the PBC.

In addition to transient synaptic adjustment involving neurotransmitters and their receptors, the cause of reduced CO activity during postnatal development may be programmed cell death in the PBC or a target area of the PBC. Apoptotic neuronal death is a critical cellular event during several stages of neuronal development. It serves to regulate the elimination of neurons that have erroneous synaptic connections or have inadequate neurotrophic factors to sustain them. In the rat brain, apoptosis reportedly occurs in two waves, around embryonic day 12 and P3, and peaks at P5 (36). Although apoptosis during early neonatal life may contribute to the plateau of CO activity at P3, it is not known whether a third wave of apoptosis occurs around P12.

NK1R mediate transmission of neurokinins, notably substance P, in the CNS. Substance P is one of the important excitatory neuromodulators in the control of respiration and is involved in the brain stem integra-

Figure 6. GABA-ir neurons in the PBC at P2 (A), P4 (B), P7 (C), P12 (D), and P21 (E). Immunoreactivity for GABA is higher at P4 and P12 than at other times. Scale bars: 20 μm.
tion of carotid body chemoreceptor reflexes (20). NK1R-ir neurons in the PBC show a striking decrease in their immunoreactivity at P12, suggesting that it may contribute to reduced CO activity there at P12.

Glu is essential for respiratory rhythmogenesis (10), the neural circuitry of central respiratory control (1), and the short-term potentiation of carotid chemoreflexes (35). Glu acts primarily at non-NMDAR within the network to generate respiratory rhythm in neonatal in vitro preparations, but it may also engage NMDAR in mature intact animals (3). Glu-ir neurons in the PBC show a small decrease in their immunoreactivity at P3–P4 and a sharp decline at P12. This may result in a depression of PBC function and contribute to a lower output to the respiratory motoneurons. Decreased excitatory activities, in turn, would contribute to reduced CO activity in the PBC at the two critical periods.

The NMDAR is an important type of GluR. Activation of NMDAR increases Ca^{2+} influx and enhances neuronal excitation. Although gene knockout studies imply that NMDAR may not be necessary for the prenatal development of circuits producing respiratory rhythm, these receptors may be important for proper postnatal development of the respiratory network in regulating respiratory rhythm (13) or in processing afferent inputs from chemosensors (29). Critical periods for NMDAR-mediated activity-dependent development occur postnatally in many systems (e.g., sensory system and spinal motoneuron circuits). NMDAR1-ir neurons in the PBC significantly reduce their immunoreactivity at P3–P4 and P12, indicating a decrease in excitatory inputs to the PBC and a transient suppression in its function. This, in turn, may contribute to the plateau and decrease in CO activity at the same time points.

GluR2 is a component of an AMPA receptor, a type of non-NMDAR. It reduces the permeability of Ca^{2+} (4) and decreases neuronal excitation. AMPA receptors include GluR1–4, and the relative levels of subunit transcription contribute strongly to the receptor’s varying properties (32). AMPA receptors are important for synaptic development, inasmuch as synapses with NMDAR, but not AMPA receptors, may be electrophysiologically “silent” (32). GluR2-ir neurons in the PBC display a minor peak at P3 and a major peak at

Fig. 7. GABA_{B} receptor (GABA_{B}R)-ir neurons in the PBC at P2 (A), P4 (B), P7 (C), P12 (D), and P21 (E). Immunoreactivity for GABA_{B}R is higher at P4 and P12 than at other times. Scale bars: 20 μm.
P12, coinciding in time with periods of low CO activity. Thus GluR2 may promote a depression of PBC functional activity at the two critical windows of development.

GABA is a major inhibitory neurotransmitter in the vertebrate CNS (26). The fast and slow actions of GABA are mediated by GABA\(_A\)R and GABA\(_B\)R, respectively (53). Tonic inhibition mediated by GABA\(_A\)R may be involved in the modulation of rhythm generation and inspiratory pattern generation (30). However, GABA content during early life may also represent an intermediate in the metabolism of glucose or fatty acids (24). GABA-ir neurons in the PBC constitute a relatively small percentage of the population, indicating that they may not play a major role in respiratory control during postnatal development. GABA-ir neurons in the PBC exhibit a developmental change during postnatal weeks 1 and 2 (an increase in LVA Ca\(^{2+}\) current with a higher threshold at P1–P3 and a decrease in LVA Ca\(^{2+}\) current with a lower threshold at P7–P15) that may contribute to the modulation of respiratory rhythm generation (33, 53). GABA\(_B\)R-ir neurons in the PBC demonstrate two peaks of immunoreactivity, at P3–P4 and P12, that implicate suppressed functional activity and decreased CO activity at these critical periods.

GlyR mediate chloride-dependent postsynaptic inhibition in cooperation with GABA\(_A\)R. However, the former plays an important role in shaping the discharge patterns of bulbar respiratory neurons necessary for respiratory rhythm generation and maintenance (39–41). GlyR-ir neurons in the PBC show two peaks of immunoreactivity, at P3–P4 and P12, suggesting a depression of PBC function and reduced CO activity at these stages.

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GABA\(_B\)R activity elicits a slow inhibitory synaptic potential by modulating low-voltage-activated (LVA) and high-voltage-activated Ca\(^{2+}\) currents in a potassium-dependent manner (53). These effects undergo a marked developmental change during postnatal weeks 1 and 2 (an increase in LVA Ca\(^{2+}\) current with a higher threshold at P1–P3 and a decrease in LVA Ca\(^{2+}\) current with a lower threshold at P7–P15) that may contribute to the modulation of respiratory rhythm generation (33, 53). GABA\(_B\)R-ir neurons in the PBC demonstrate two peaks of immunoreactivity, at P3–P4 and P12, that implicate suppressed functional activity and decreased CO activity at these critical periods.

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Neonatal and adult animals may have different synaptic mechanisms in the PBC. The blockage of chloride-dependent inhibition with GlyR antagonists effectively stops regular respiratory rhythmogenesis in
Fig. 9. Optical densitometric measurements of CO reaction product (A) and immunoreaction product for Glu (C), NMDAR1 (D), GluR2 (E), GABA (F), GABAr (G), and GlyR (H) in the PBC during postnatal development. ANOVA of each marker (A and C–H) and of neuronal areas in the PBC (B) showed significant differences among ages (P < 0.01). B: steady increase in mean neuronal areas in the PBC during postnatal development. Statistical comparisons (Tukey's Studentized range test) between successive age groups: *P < 0.05; **P < 0.01 (significance between one age group and its adjacent younger age group).
adult animals but not in neonates (31). In contrast, the blockage of non-NMDAR in the neonate abolishes respiratory rhythm (14), while the blockage of non-NMDAR and NMDAR is required for the same effect in the adult (11). Our previous study indicates that synaptic interactions within the PBC of adult rats involve a variety of neurotransmitter and receptor types, such as Glu, GABA, and Gly and their receptors (18). The present study suggests that, during postnatal development, inhibitory inputs (mediated by GABA, GABAR, and GlyR) and excitatory inputs (mediated by Glu, NMDAR, and substance P) may coordinate to affect PBC’s functions and maturation. An imbalance of excitatory and inhibitory synapses favoring the latter may contribute to the two critical periods of low CO activity (P3–P4 and P12). Of special note is that the change at P12 for CO and all neurochemicals examined is much more pronounced than that at P3–P4, indicating that P12 may represent a period of greater synaptic adjustment and, perhaps, greater vulnerability to respiratory failure.

Sudden infant death syndrome (SIDS) is a common cause of infant mortality between 1 wk and 1 yr of age, especially between 1 and 4 mo of age. The cause of SIDS remains poorly understood. Previous studies suggest that apnea leading to SIDS may result from a prone sleeping posture, a delayed brain stem maturation (e.g., a higher dendritic spine density in brain stem nuclei that persists longer than that of age-matched controls) (46), a delayed myelination in the CNS (16), a high rate of apoptosis in the brain (48), regional ischemia of the brain stem, or other factors. On the basis of our present and previous studies (19), we postulate that during the critical periods of postnatal development, when respiratory nuclei are under a stronger inhibitory drive (P3–P4 and especially P12 in the rat), respiratory distress may pose an overwhelming burden on the already vulnerable brain stem nuclei, leading to abnormal respiratory control and perhaps death. Synaptic imbalance may be one of the intrinsic factors related to SIDS, when combined with respiratory insults.

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REFERENCES


