Developmental study of cytochrome oxidase activity in the brain stem respiratory nuclei of postnatal rats

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Liu, Ying-Ying, and Margaret T. T. Wong-Riley. Developmental study of cytochrome oxidase activity in the brain stem respiratory nuclei of postnatal rats. J Appl Physiol 90: 685–694, 2001.—We utilized cytochrome oxidase (CO) as a marker of neuronal functional activity to examine metabolic changes in brain stem respiratory nuclei of rats from newborn to 21 day of age. The pre-Botzinger complex (PBC), upper airway motoneurons of nucleus ambiguus (NAUAM), ventrolateral nucleus of solitary tract (NTSvl), and medial and lateral parabrachial nuclei (PBM and PBL, respectively) were examined at postnatal days (P) 0, 1, 2, 3, 4, 5, 7, 14, and 21. CO histochemistry was performed, and the intensity of CO reaction product was quantitatively analyzed by optical densitometry. In addition, CO histochemistry was combined with neurokinin-1 receptor (NK1R) immunogold-silver staining to doubly label neurons of PBC in P14 animals. The results showed that levels of CO activity generally increased with age in all of the nuclei examined. However, a significant decrease in CO activity at P3 (% P < 0.01), and a distinct plateau of CO activity was noted at P3 in PBC and at P3 and P4 in NTSvl, PBM, and PBL. Of the neurons examined in PBC, 83% were doubly labeled with CO and NK1R. Of these, CO activity was high in 33.9%, moderate in 27.3%, and light in 38.8% of neurons, suggesting different energy demands in these metabolic groups that may be related to their physiological or synaptic properties. The transient decrease or plateau in CO activity at P3 and P4 implies a period of synaptic adjustment or reorganization during development, when there may be decreased excitatory synaptic drive or increased inhibitory synaptic drive, or both, in these brain stem respiratory nuclei. The adjustment, in turn, may render the system less responsive to respiratory insults. This may bear some relevance to our understanding of pathological events during postnatal development, such as occurs in sudden infant death syndrome.

CO histochemistry; cytochrome oxidase; postnatal development; upper airway motoneurons; NK1R immunoreactivity; brain stem respiratory nuclei; pre-Botzinger complex; dorsal respiratory group (DRG); ventrolateral nucleus of solitary tract (NTSvl); pontine respiratory group (PRG); medial and lateral parabrachial nuclei (PBM and PBL, respectively) and Kålliker-Fuse nucleus (1, 27). Three basic types of respiratory neurons have been identified: inspiratory neurons, expiratory neurons, and phase-spanning neurons (1, 27). Extensive connections among the three respiratory groups have been mapped in the rat. Thus, after injections of fluorescent dyes into VRG, retrogradely labeled neurons have been found in DRG and PRG, and anterogradely labeled terminals were traced to DRG and PRG as well as down to the spinal cord (15, 33). Moreover, labeled efferent terminals were closely apposed to the somata and dendrites of labeled neurons in DRG and PRG, suggesting monosynaptic connections between the VRG and these neurons (33). Recently, in vitro brain stem slice preparations from neonatal rodents have provided supporting evidence to the postulate that PBC is the center or kernel for the generation of respiratory rhythmicity (6, 19, 22, 26). However, most of the previous studies were based mainly on physiological and histological approaches, and they only circumscribed the approximate boundaries of the PBC, because it lies in the heterogeneous reticular formation of ventrolateral medulla oblongata. More recently, the anatomic structure of the PBC has been defined in adult female rats and mice by the presence of neurokinin-1 receptor (NK1R) immunoreactivity, implying that the NK1R immunoreactive neuronal somata in the ventrolateral medulla oblongata delineate the precise anatomic feature of the PBC (7).

Cytochrome oxidase (CO) is the terminal enzyme in the mitochondrial respiratory chain and is a vital component of cellular energy production and transduction. In the brain, regional CO activity provides an index of local tissue energy metabolism, which is tightly coupled to neuronal functional activity, especially of synaptic and spontaneous types (30, 31). The level of CO activity in a neuron is most closely correlated with ion pumping for the reestablishment of the resting membrane potential after excitatory postsynaptic depolarization.
ization (30, 31). Therefore, we used CO as a marker of neuronal activity to demonstrate functionally related metabolic characteristics in neurons of NTSVL, PBm, PBp, and upper airway motoneurons of NA (NAuAM) in rats from newborn to 21 days of age. In addition, we used NK1R antibody to define the PBC in all age groups of animals. Furthermore, we combined CO histochemistry and NK1R immunohistochemistry to doubly label CO activity and NK1R expression in PBC of postnatal day (P) 14 rats. By conducting an in-depth developmental study of CO activity in the brain stem respiratory nuclei of postnatal rats, we hope to establish baseline data for future studies on experimentally induced respiratory distress. Because brain stem developmental abnormality is implicated in sudden infant death syndrome (SIDS) (4), a basic understanding of the developmental activities of these nuclei is of considerable interest.

**MATERIALS AND METHODS**

**Tissue preparation.** All animals in this study were used in accordance with National Institutes of Health and Medical College of Wisconsin regulations. A total of 88 rats were used in 9 groups (n = 8 in each group) of ages P0, 1, 2, 3, 4, 5, 7, 14, and 21. For CO histochemistry, the animals were deeply anesthetized with ether and perfused transcardially with 2% paraformaldehyde and 2% glutaraldehyde in 4% sucrose and 0.1 M sodium phosphate buffer, pH 7.4. For NK1R immunohistochemistry and double labeling with CO histochemistry and NK1R immunogold-silver staining (IGSS), the animals were perfused with 4% paraformaldehyde in 4% sucrose and 0.1 M sodium phosphate buffer, pH 7.4. The brain stems were then removed and postfixed by immersion in the same fixative as for perfusion for 3 h at 4°C. They were then cryoprotected in increasing concentrations of sucrose (10, 20, and 30%) in 0.1 M sodium phosphate buffer 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 20-μm thickness on a freezing sliding microtome. Alternate sections were either reacted histochemically for CO activity or stained with cresyl violet. The basic protocol for CO activity was as described previously (29). Briefly, the sections were incubated in 0.1 M sodium phosphate buffer, pH 7.4, containing 25 mg 3,3′-diaminobenzidine (DAB; Sigma), 10 mg cytochrome c, and 2% sucrose per 50 ml of solution. Incubations were carried out at 37°C in the dark for exactly 3 h. Sections from different age groups were reacted together to avoid any differences due to slight variations in temperature, medium composition, or incubation time. After incubation, the sections were washed three times for 5 min in cold 0.1 M sodium phosphate buffer and mounted on gelatin-coated slides. The slides were then air dried and coverslipped.

**Fig. 1.** Low-magnification photomicrographs showing pre-Bötzing complex (PBC) and nucleus ambiguus (NA) in coronal sections of the brain stem of postnatal day (P) 14 rats. Alternate sections were labeled with neurokinin-1 receptor (NK1R) antibody in A or reacted for cytochrome oxidase (CO) histochemistry in B. Insets in A and B: higher magnifications of labeled neurons in the relevant circled areas with asterisks. C: NK1R-immunoreactive neurons in PBC (arrows) and NA (arrowheads). Dashed line roughly delineates the boundary between NA to the upper right and PBC to the lower left. The plasma membrane of neurons and processes are intensely stained with NK1R in C, D, and E (arrows and arrowheads). At times, the cell bodies in PBC are also lightly labeled with NK1R (arrows in C). Thin and long processes in PBC (C) extend dorsally to the NA and ventrally to the ventral reticular formation. D and E: neurons of PBC doubly labeled with CO and NK1R. NK1R-immunogold-silver particles highlight the cell surfaces and processes (arrows). Neurons dartyly, moderately, or lightly reactive for CO activity are denoted by D, M, and L, respectively (B inset, D, and E). As mentioned in **MATERIALS AND METHODS**, sections for double labeling were incubated for a shorter time than that for CO histochemistry alone so as not to obscure silver labeling. Thus intensities of CO reaction product in D and E are generally lower than those in B (inset). Nevertheless, a range of intensities from light through moderate to dark can still be discerned.
NK1R immunohistochemistry and CO histochemistry/NK1R-IGSS double labeling. Coronal sections of frozen brain stems were cut at 12-μm thickness on a cryostat. Alternate sets of serial sections were mounted on gelatin-coated slides and then reacted for NK1R immunoreactivity or for CO histochemistry and NK1R-IGSS double labeling, respectively.

For NK1R immunohistochemistry, the slides were incubated in anti-NK1R polyclonal antibodies (Sigma) diluted at 1:15,000 in PBS for 36–48 h at 4°C; this was followed by incubation with goat anti-rabbit IgG-horseradish peroxidase for 4 h at room temperature. The reaction was detected by immersing the slides in DAB solution for 5–10 min and then stopped by cold PBS. The slides were then dehydrated and coverslipped.

CO histochemistry and NK1R-IGSS double labeling was performed by incubating the slides first for CO histochemistry at 37°C for 2 h (instead of 3 h as described above) and then for NK1R-IGSS. A less intense histochemical reaction enabled a clear distinction between reaction products of CO and of NK1R. As described previously (11) with minimal modification, CO-reacted slides were blocked in 10% normal goat serum in PBS, pH 7.4, overnight at 4°C. Anti-NK1R antibody was diluted to 1:15,000 in 5% normal goat serum and applied to slides for 36–48 h at 4°C. After incubation with immunogold (goat anti-rabbit IgG, 5-nm gold conjugate) diluted at 1:100 for 4 h at room temperature, signals were detected by a silver-enhancing kit (BBInternational) for 8–10 min at room temperature in the dark. Between steps, slides were rinsed in PBS three times for 5 min. Before and after silver enhancing, the slides were rinsed with distilled water. The slides were then dehydrated and coverslipped.

Cell size measurements. Neurons of PBC, NAuAM, NTSVL, PBn, and PBt at various ages were measured for cell size in cresyl violet-stained sections adjacent to those reacted for CO. The long and short axes of neurons were measured under the microscope, using a ×40 objective and a reticule. The average diameter was calculated, and the cell areas were determined.

Quantitative densitometry. CO-reacted sections were analyzed with a Zeiss Zonax MPM 03 photometer system with a ×25 objective and a 2-μm-diameter measuring spot. White (tungsten) light was used for illumination, and lighting conditions were held constant for all of the measurements.

Fig. 2. Postnatal cell size changes (A) and optical densitometric measurements of CO reaction product (B–D) in neurons of PBC in rats between P0 and P21 of age. There is a steady increase in cell size with age. A distinct plateau of CO activity was noted at P3. Statistical comparisons were made between one age group and the next older group as marked at the bottoms of columns. *P < 0.01, **P < 0.001; ns, no significant difference.
Because the white matter exhibited very low levels of CO activity, it was used as an internal standard for our study; hence, the white matter was subtracted as the background and was set to zero over each section examined. Optical density readings were performed on neuronal perikarya in sections bearing the nuclei examined, and the optical density of each cell was an average reading of two to four regions of its cytoplasm. Twenty to sixty cells in each nuclear group (depending on the size of the nuclei) for each animal, a total of 135–377 cells for each nuclear group at each age were measured. Mean optical densities and standard deviations of each cell type at each age were then obtained. Statistical comparisons were made between successive age groups (CO activity in P0 vs. P1, P1 vs. P2, and so on). Statistical significance was determined by using both one-way ANOVA to control the type I comparisonwise error rate and Tukey’s Studentized range test to control the type I experimentwise error rate, general linear models procedure (SAS system). Significance level was set at <0.01 for one-way ANOVA and <0.05 for Tukey. Variations in neuronal areas and CO optical density between P2 and P4 were determined. For example, variations between P2 and P3 were calculated by the following formula: \( \frac{P3 \text{ (areas or optical density)} - P2 \text{ (areas or optical density)}}{P2 \text{ (areas or optical density)}} \). The same was done for variations between P3 and P4. Pearson correlation coefficients on variations between neuronal areas and CO optical density were performed in all nuclei examined between P2 and P4 rats. A \( P \) value of 0.01 or less is considered significant.

**RESULTS**

Characteristics of neurons in the brain stem respiratory nuclei studied. NK1R-immunoreactivity precisely delineated two groups of nuclei in the ventrolateral medulla oblongata of rats from P0 to P21 of age: PBC was localized ventrally and NA dorsally (Fig. 1A). They were also clearly identified in adjacent CO histochemical sections (Fig. 1B). CO-reactive neurons of PBC exhibited dark, moderate, or light intensities of CO labeling (Fig. 1B). NK1R immunoreactivity was mainly expressed along the plasma membrane of cell bodies and processes of neurons (Fig. 1, A, C, D, and E). At times, the cytoplasm of neurons was also slightly labeled with NK1R (Fig. 1C). NK1R-immunoreactive neurons of PBC were round, oval, or multipolar in shape and small to medium in size and were dispersed among the ventral reticular formation. Cell sizes between P0 and P21 are shown in Fig. 2. Thin and long processes of PBC often traversed among neurons of the PBC, toward the midline of the brain stem, or extended dorsally to the NA and ventrally to the ventral reticular formation (Fig. 1C). In sections doubly reacted for CO histochemistry and NK1R-IGSS, the immunoreactive silver particles clearly highlighted the surfaces of neurons in PBC, whereas the cytoplasm of neurons showed dark, moderate, or light intensities of CO labeling (Fig. 1, D and E). In such a series of doubly reacted sections (every third section through the medulla) from a P14 rat, we found that 83% of PBC neurons (121/146) were doubly labeled with CO and NK1R. Of these doubly labeled neurons, 33.9% (41/121) showed high levels of CO activity, 27.3% (33/121) moderate, and 38.8% (47/121) low levels. In comparing NK1R immunoreactive processes between PBC and NA, thin long processes were often found among neu-

![Fig. 3. Low-magnification photomicrographs of coronal sections of the brain stem from P14 rats. Alternate sections were stained for cresyl violet in A and C or reacted for CO in B and D. Neurons in NA and ventrolateral nucleus of the solitary tract (NTS_{vl}) are shown in A and B and medial and lateral parabrachial nuclei (PBm and PBl, respectively) in C and D. Insets show higher magnifications of the relevant circled areas.](http://jap.physiology.org/)
rons of PBC, whereas in NA, NK1R immunoreactive boutons could easily be seen contacting the cell surfaces (Fig. 1C).

Neurons of NA include motoneurons that are distributed mainly in the dorsal division and interneurons that are localized in the ventral division or between the motoneurons. Esophageal motoneurons are found in the rostral pole of NA, pharyngeal and cricothyroid motoneurons in the intermediate portion, and laryngeal motoneurons in the caudal pole of NA (8). Of these, the last two groups of motoneurons contribute to the control of upper airway respiratory muscles (21) and are referred to here as NAUAM. Our cell size measurements and CO optical density readings were carried out on NAUAM. Interneurons of NA that are usually oval, fusiform, or pyramidal in shape and smaller than motoneurons are thought to include not only respiratory interneurons but also other visceral efferent preganglionic neurons, so they are not under consideration in this study. Neurons of NAUAM were pyramidal or multipolar in shape and were medium to large in size in cresyl violet-stained sections (Fig. 3A). Cell sizes between P0 and P21 were plotted in Fig. 4.

CO reaction product was present in NAUAM between P0 and P21 (Figs. 3B and 5) and was relatively intense even at P0 (Fig. 5A). CO reaction product was distributed unevenly within the cytoplasm, with the highest intensity generally surrounding the nucleus and the lowest along the periphery of the cell body extending into the dendrites (Figs. 3B and 5, B, D, and F).

Neurons of NTSvl, PBm, and PBl, as seen in cresyl violet-stained sections were round, oval, fusiform, pyramidal, or multipolar in shape (Fig. 3, A and C) and small to medium in size. Cell sizes between P0 and P21 were plotted in Fig. 4. CO reaction product was found in all three nuclei at all ages examined (Figs. 3, B and D, 6, and 7). Of the various subgroups of NTS, neurons of NTSvl showed the most intense CO activity at all age groups examined (Fig. 3B). The neuropil in NTSvl and PBl had moderate to high levels of CO activity (Figs. 6 and 7).

CO optical densitometry. Optical densities of CO reaction product in neurons of PBC, NAUAM, NTSvl, PBm, and PBl were taken at each age group between P0 and P21 and were plotted in Figs. 2 and 8. In all of the nuclei examined, levels of CO activity showed a

![A Upper Airway Motoneurons of NA](image1)

![B Ventrolateral N. Solitary Tract](image2)

![C Medial Parabrachial N.](image3)

![D Lateral Parabrachial N.](image4)

Fig. 4. Postnatal cell size changes in the upper airway motoneurons of NA (NAUAM; A) and the neurons of NTSvl (B), PBm (C), and PBl (D) in rats between P0 and P21 of age. N, nucleus.
general increase from P0 to P21. However, the trend was interrupted by a plateau or a transient decrease of CO activity at P3 in PBC and P3 and P4 in NAUAM, NTSVL, PBM, and PBL. A significant decrease in CO activity was found in NAUAM (P < 0.01). A plateau of CO activity (indicating no significant decreases or increases) was found in NTSVL, PBM, and PBL at P3 and P4 of age. Tukey’s Studentized range test yielded results that were consistent with one-way ANOVA (see Table 1). To examine the possibility that the increase in CO activity with development was simply related to an increase in cell size, the Pearson correlation coefficient analysis between neuronal areas and CO optical densities was done. The results showed no statistically significant correlation between CO levels and cell size in all of the nuclei examined between P2 and P3, and in PBC, NAUAM, NTSVL, and PBM between P3 and P4. It reached a significance in PBL between P3 and P4 (P < 0.01).

**DISCUSSION**

CO serves as a reliable indicator of neurons’ oxidative capacity and energy metabolism. The tight coupling between energy metabolism and neuronal activity further enables CO to serve as a sensitive metabolic marker for neuronal functional activity. The levels of CO activity in a neuron can vary independent of the cell size or neurotransmitter synthesis but are closely related to the proportion of excitatory synaptic input received by the neuron (12, 14). This suggests that the level of CO activity in a neuron is most closely correlated with ion pumping for the reestablishment of the resting membrane potential after excitatory postsynaptic depolarization (30, 31). To a certain extent, the level of CO in a neuron is also related to its sustained firing rate generated synaptically or spontaneously (9). Other processes, such as cell growth or neurotransmitter synthesis, make lesser demands on a neuron’s oxidative metabolic machinery (30, 31). For example, granule cells, which have high rates of division and growth postnatally, have low levels of CO, lactate dehydrogenase, and succinate dehydrogenase activity (12, 32). Cerebral oxygen consumption is low at birth and peaks at the time of synaptic maturity (3). During postnatal development, cerebellar Purkinje cells transiently receive incoming excitatory climbing fiber input. This is the time when their cell bodies exhibit the highest level of CO activity (12). When the climbing fibers move up to the dendrites and leave the cell bodies with exclusively inhibitory basket cell contacts, the level of CO activity in Purkinje cell bodies drops, even though the neurons continue to enlarge in size (12). Thus levels of CO activity in the nuclear groups examined in the present study are likely to reflect the degree of excitatory synaptic input during postnatal development rather than cell growth or protein synthesis.
The PBC has been postulated to be the center for the generation of respiratory rhythmicity (6, 19, 22, 26). This presumably involves intrinsic activities as well as synaptic activities from excitatory and inhibitory inputs (19, 20, 22, 25). There are different types of respiratory neurons in PBC with varying depolarization patterns and timing (1, 19, 23). They are likely to receive different ratios of excitatory to inhibitory inputs, which may place varying demands on their energy metabolism. Indeed, PBC neurons in the present study exhibit heterogeneous levels of CO activity.

NTSVL is thought to be mainly involved in respiratory modulation. Although NTSVL in the rat contains fewer respiratory-related neurons than in the cat, it indeed receives respiratory-relevant information from the lung, notably from the slowly adapting pulmonary stretch receptors (2) and from the carotid body chemoreceptors (5). Moreover, neurons of NTSVL may receive terminal inputs from the rostral VRG and project, in turn, back to the rostral VRG (33). In the present study, NTSVL exhibited the highest level of CO activity among the subgroups of NTS, suggesting that respiratory control imposes a greater demand on energy metabolism than some of the other functions performed by NTS.

No doubt, NTSVL also serves other functions besides modulating respiration, and even the preinspiratory neurons in PBC fire during nonrespiratory activity (34). Because the respiratory nuclei examined in this study are heterogeneous pools involved in a variety of tasks in addition to respiration, we cannot rule out the possibility that the changes we observed are related to nonrespiratory neurons within the nuclei with nonrespiratory-related activity. However, the fact that CO activity follows a consistent pattern in all major respiratory nuclei, including those that are more homogeneous than others (NTSVL), suggests that the changes described here do reflect primarily changes in functions related to respiration.

The neuropil in NTSVL and PB_L showed high levels of CO activity. Neuronal processes intermingled with each other and were in close contact with neuronal cell bodies. NTSVL is thought to make little or no contribution to the respiratory drive transmitted from the medulla to the spinal motoneurons (1). Nevertheless, NTSVL remains an important relay for integrating respiratory afferent inputs as mentioned above. Such integrative activities may contribute to high levels of energy metabolism in the neuropil of NTSVL. The same is true for PB_L. Neurons in PB_L are not essential for respiratory rhythm generation, but they stabilize respiratory pattern, slow rhythm, and influence the timing of respiratory phase (1). These activities may again account for high CO levels in the neuropil of PB_L in gathering and integrating information and controlling respiration.

Fig. 6. A–F: CO-reactive neurons in NTSVL of rats at P0, P2, P3, P7, P14, and P21, respectively (arrows). The neuropil shows an increase in the level of CO activity with age.
CO optical density readings showed a general increasing trend of CO activity with age in neurons of PBC, NAuAM, NTSvl, PBM, and PB_L between P0 and P21. The increase in CO activity in the respiratory nuclei examined implicates an increase in depolarization-induced ion-pumping activity and synaptic functions during postnatal life in rats. Of particular interest is a consistent plateau or transient decrease in the level of CO activity within a narrow window (P3 for PBC and P3 and P4 for the other nuclei examined) that was not correlated with changes in cell size. There may be two possibilities for the decrease in CO activity: 1) neuronal regression or normal neuronal death by apoptotic mechanism and 2) decrease in neuronal function or synaptic excitation. Neuronal regression during development is at least as important as neuronal growth and occurs in a variety of brain areas, including the brain stem (13). The partial loss of neurons or their axons from an initial population serves to eliminate erroneous connections and is probably an adjustment to a target field in which the axons compete for a limited amount of growth-controlling trophic factors (13). In the developing rat brain, apoptosis occurs from gestational day 12 to P5. It appears that there are two waves of apoptotic process during development. One is around gestational day 12, and the other starts around P3 and peaks at P5 (17). The postnatal apoptotic window corresponds closely to the P3–P4 window of the present study. Thus the apoptotic process may contribute in a major or minor way to the plateau or decrease in CO activity postnatally. Alternatively, of more important consideration is synaptic adjustment or a change in synaptic activation. A decrease in excitatory drive (either in the number and/or the frequency of synapses), an increase in inhibitory drive, or both would place a lower demand on energy metabolism of these neurons, hence producing a decrease in CO activity at P3 and P4. This would have direct bearing on respiratory generation and modulation. Therefore, if the changes in CO activity reflect synaptic adjustment or reorganization, this may represent a period when the system is less responsive to changing respiratory demands and, therefore, more vulnerable to perturbation and insults. Indeed, an increase in inhibitory synaptic mechanism in respiratory modulation between the first postnatal days of life and older ages has been postulated in rat (24) and mouse (16), based on both in vivo and in vitro physiological studies. For example, the occurrence of inhibitory postsynaptic potentials in neurons of NTS is much lower at P0–P3 of rats than those at P4–P5. It reaches the adult level between P6 and P9, suggesting that a maturational step occurred in the NTS at P4 and P5 of age (24).
An increase in inhibitory synaptic activity, however, does not necessarily imply a decrease in neuronal CO activity if the excitatory drive is also enhanced. As discussed above, the adjustment of a neuron's CO activity is strongly influenced by the ratio of excitatory and inhibitory inputs it receives. As respiratory neurons acquire more inhibitory synapses after P4, their excitatory inputs are likely to be strengthened as well so that the overall effect after P4 may be a steady increase in CO activity. In the present study, we have not focused on the day-by-day changes in neuronal CO activity between P8 and P13, as well as between P15 and P20; hence, it remains unknown whether there is another decrease or plateau of CO activity between P8 and P21. However, our finding of such a change in CO activity at P3 and P4 strongly implicates synaptic adjustment or reorganization at this critical time of development.

SIDS is the most common cause of infant death between 1 wk and 1 yr of age. The peak is between 1 and 4 mo of age, with few deaths in the first few weeks or after 6 mo of life (10). Although the main cause of SIDS induction remains poorly understood, growing evidence suggests a brain stem developmental abnormality. For example, a higher dendritic spine density in brainstem nuclei of SIDS than of age-matched controls implies a delayed brain stem maturation (18).

Table 1. Tukey's Studentized range test for analysis of CO activity in nuclei examined between successive age groups in rats

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<th>Successive Age Groups</th>
<th>PBC†</th>
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*P, postnatal day; PBC, pre–Bötzinger complex. †: the same level of significance among high, medium, and low cytochrome oxidase (CO) activity of neurons in PBC; NA_{UAM}, upper airway motoneurons of nucleus ambiguus; NTS_{VL}, ventrolateral nucleus of solitary tract; PB_{M} and PB_{L}, medial and lateral parabrachial nuclei, respectively. *P < 0.05 of comparison significance; ns, no significant difference; ↓, decrease in CO activity.
A higher rate of apoptosis has been reported in the brains of SIDS cases than that of control groups, and the highest occurrence was found in the brain stem, including NTS (28). Whether the highest death rate in SIDS between 1 and 4 mo reflects a vulnerable time during the postnatal developmental period is not known at this time. The present finding of a transient reduction or plateau of CO activity in the brain stem respiratory nuclei at early stages of postnatal development is consistent with a vulnerable period when the system may be less responsive to respiratory insults. This may bear some relevance to our understanding of pathological events during postnatal development, such as occurs in SIDS.

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