Decreased energy metabolism in brain stem during central respiratory depression in response to hypoxia

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LaManna, J. C., M. A. Haxhiu, K. L. Kutina-Nelson, S. Pundik, B. Erokwu, E. R. Yeh, W. D. Lust, and N. S. Cherniack. Decreased energy metabolism in brain stem during central respiratory depression in response to hypoxia. J. Appl. Physiol. 81(4):1772–1777, 1996.—Metabolic changes in the brain stem were measured at the time when oxygen deprivation-induced respiratory depression occurred. Eucapnic ventilation with 8% oxygen in vagotomized urethane-anesthetized rats resulted in cessation of respiratory drive, monitored by recording diaphragm electromyographic activity, on average within 11 min (range 5–27 min), presumably via central depressant mechanisms. At that time, the brain stems were frozen in situ for metabolic analyses. By using 20-µm lyophilized sections from frozen-fixed brain stem, microregional analyses of ATP, phosphocreatine, lactate, and intracellular pH were made from 1) the ventral portion of the nucleus gigantocellularis and the parapyramidal nucleus; 2) the compact and ventral portions of the nucleus ambiguous; 3) midline neurons; 4) nucleus tractus solitarii; and 5) the spinal trigeminal nucleus. At the time of respiratory depression, lactate was elevated threefold in all regions. Both ATP and phosphocreatine were decreased to 50 and 25% of control, respectively. Intracellular pH was more acidic by 0.2–0.4 unit in these regions but was relatively preserved in the chemosensitive regions near the ventral and dorsal medullary surfaces. These results show that hypoxia-induced respiratory depression was accompanied by metabolic changes within brain stem regions involved in respiratory and cardiovascular control. Thus it appears that there was significant energy deficiency in the brain stem after hypoxia-induced respiratory depression had occurred.

ventral medulla oblongata; intracellular pH; adenosine 5'-triphosphate; phosphocreatine; lactate

HYPOXIA INDUCES increased respiration through activation of peripheral chemoreceptor reflex pathways. However, in the absence of afferent inputs from peripheral chemoreceptors, O2 deprivation causes depression of breathing activity in anesthetized animals. Evidence of hypoxic depression of respiration also exists in animals and in humans with intact peripheral chemoreceptors (28). The effects of central hypoxia on respiratory activity require more time to develop than do the changes induced by hypoxic stimulation of breathing through peripheral chemoreceptors, suggesting the involvement of relatively slow metabolic processes. The mechanism by which hypoxia causes respiratory depression is not known, but several possibilities have been suggested (28).

One possibility is that increased tissue lactate is responsible for respiratory depression. Decreased O2 availability promotes the metabolic production of lactic acid by glycolysis within the cells. The lactic acid may then diffuse into the extracellular fluid, or cells may actively transport H+ outward. Hypoxic lactic acidosis might act indirectly through inhibition of glutamate receptors (11, 35). The N-methyl-D-aspartate (NMDA)-receptor pathway plays a significant role in cardiovascular and respiratory activity including respiratory timing (4), and pH-induced changes in the NMDA-receptor function might partly mediate hypoxia-induced respiratory modulation.

Although cell ATP concentrations are buffered by phosphocreatine (PCr), if hypoxia is prolonged and severe, a decrease in cellular ATP will occur. ATP can either directly modulate ion channels as a ligand or play a role in channel modulation by phosphorylation. Recently, it has been shown that excitability of neurons could be altered via Na+-channel modulation as well as by modulation of ATP-sensitive K+ channels (16, 17), which promote hyperpolarization when open. The closure of these channels is ATP dependent, and the channel affinity for ATP is pH dependent, decreasing with increasing acidosis (9). Thus higher concentrations of ATP would be required to keep the channel closed in acidic conditions.

Whether hypoxia-induced decreases in PCr and ATP occur and whether these changes are closely related in the brain stem nuclei involved in breathing and cardiovascular control are not known. Furthermore, the relationships among regional alterations in intracellular pH (pHi), PCr, and ATP are not well established. Despite the importance of pH, and of energetic sources in brain function, regional changes in these variables in relation to respiratory depression induced by O2 deprivation have not been studied. This has been partly due to the lack of quantitative methods for their simultaneous measurements.

To test the hypothesis that metabolic changes within the brain stem in response to hypoxia are coordinated processes that play an important role in the global behavior of respiratory and cardiovascular outputs, we determined the specific regional metabolic response patterns within multiple regions of the medulla oblongata to decreased fractional inspiratory O2 under eucapnic conditions. A preliminary report of these data has already appeared (22).

MATERIALS AND METHODS

Animals and surgical preparation. Eighteen male Sprague-Dawley rats were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee. Each rat was anesthetized with urethan (1.2 g/kg ip). A femoral artery was cannulated to measure arterial pressure and heart rate and to withdraw samples for blood gas analyses. The external jugular vein was cannulated for administration of fluid and drugs. All rats were tracheo-
mized and ventilated with 100% O₂. The volume and the pump rate were adjusted to the body weight and breathing frequency. The body temperature of the animals was maintained at 37–38°C. To expose the ventrolateral medulla, a ventral midline incision was made in the neck. The esophagus and the trachea were transected, and the proximal portions of both were reflected cranially. After removal of overlying muscles, a wide cranietomy was performed, the ventral aspect of the medulla was exposed, and the intact dura mater was covered with warm artificial cerebrospinal fluid, as described earlier (14). The vagus nerves were isolated and cut to eliminate input from lung mechanoreceptors and prevent eventual entrainment of respiratory output to the ventilator.

Respiratory output was obtained by recording the electromyographic activity of the diaphragm (EMGdi), as earlier described (14). Briefly, bipolar stainless steel twisted wires, with wire tips 1 mm apart, were implanted via an abdominal incision, which was later sutured closed, into the costal part of the diaphragm near the lateral position of the central tendon. The electrical activity was amplified with an AC-coupled amplifier having a band-pass filter setting of 3 Hz to 3 kHz (model PH 511, Grass Instruments). The signal was full-wave rectified and then processed by a moving averager with a 100-ms time constant. EMGdi activity was recorded simultaneously with blood pressure on a six-channel Gould strip-chart recorder. The rate of ventilation was adjusted to

\[
\text{pH} = \left( \frac{\text{absorbance}_{550}}{\text{absorbance}_{450}} \right) - 10.5 / 1.3 \quad (1)
\]

Values (pH) are reported as means ± SE from investigator-selected regions of interest or as histogram distributions of individual pixels, each ~0.01 × 0.01 mm in size. Details of the methods and review of the physical, chemical, and biological characteristics of neutral red have been documented (19, 21, 23). Briefly, the dye is taken up rapidly from the vascular compartment into the brain and is found to be concentrated in all the cell types in the cytoplasmic organellar membranes, where it is responsive to the cytoplasmic compartment. The cell membrane, nucleus and nuclear membrane, and myelin remain unstained. This is a ratio method and is concentration independent as long as a minimum amount of dye is present. The method is quantitative with respect to pH over the range of at least 6.0–8.0.

Metabolite assays. Twenty-micrometer sections, cut at −24°C, of the frozen brains were collected and lyophilized for metabolite determinations. These sections were cut from the block face of the frozen brain stem just after the block face was photographed for pH determination by neutral red. Thus the regions sampled for metabolic assay corresponded directly to the regions in which pH was determined. Lactate, PCr, and ATP were determined by microquantitative histochemistry on the freeze-dried sections as described previously (25, 26). Discrete samples, corresponding to the regions of interest used for pH analyses, two samples per region, were dissected in a low-humidity room with the aid of a dissection microscope. The 0.3- to 4.3-μg (1.5 ± 1.0 SD) tissue samples were weighed on a quartz-fiber balance. The tissue samples were assayed for ATP and PCr by using the luciferin-luciferase method; lactate was assayed by using enzymatic cycling.

Fig. 1. Diaphragm electromyogram (EMGdi) recorded from a rat exposed to continuous hypoxia beginning at left arrow. Activity is observed initially to increase in frequency, then strength. A prolonged slowing follows until strength drops to zero. Brain stem was frozen in situ at indicated time (right arrow).
Regions of interest. The brain stem was sectioned coronally to a level 12–13 mm posterior to the bregma (i.e., between the pontomedullary border and the calamus scriptorius). Regions of interest were defined as 1) the ventral portion of the nucleus gigantocellularis and the parapyramidal nucleus; 2) the compact and ventral portions of the nucleus ambiguus; 3) midline neurons; 4) the nucleus tractus solitarii; and 5) the spinal trigeminal nucleus.

Data analyses and statistics. O2 saturation was calculated from the measured arterial Pao2 (Pao2) samples on the basis of the O2 half-saturation pressure of hemoglobin = 36 Torr for Sprague-Dawley rats (5) and a Bohr coefficient = -0.52 (18).

The results of the metabolite assays were analyzed for statistical significance by using analysis of variance (ONE-WAY procedure of SPSS for Windows, version 5). After significance between the hypoxic and control groups with respect to each metabolite was ascertained, pairwise comparisons were made between comparable regions by independent-sample two-tailed t-test. All data are reported as means ± SE, except where indicated as SD. The number of observations used for each comparison is indicated in the text and Table 1.

RESULTS

At the time when hypoxic-induced respiratory depression had occurred after the inspired gas was switched from O2 to the hypoxic gas mixture (8% O2 in N2), there was an expected reduction in Pao2 (411 ± 21 vs. 35 ± 3 Torr; n = 11, P < 0.01), no change in Paco2 (33 ± 3 vs. 34 ± 3 Torr; n = 11, P > 0.1), and significant fall in pH (7.42 ± 0.02 vs. 7.23 ± 0.02; n = 11, P < 0.05). This hypoxic Pao2 corresponds to an O2 saturation of ~38%. As shown in Fig. 1, exposure to isocapnic hypoxia caused an initial increase in respiratory frequency, followed by an increase in amplitude and then decrease in rate of breathing, reduction of peak activity, and apnea. At the time of freezing, the mean systemic arterial blood pressure was 60 ± 6 mmHg, which was lower than the prehypoxic blood pressure of 91 ± 6 mmHg (n = 8, P < 0.01). Six rats were frozen in situ under control conditions: PaO2 = 430 ± 11 Torr, PaCO2 = 38 ± 2 Torr, pH = 7.36 ± 0.02, and mean arterial pressure = 87 ± 7 mmHg. The blood gas values and pH were not significantly different from the prehypoxic blood gases and pH of the hypoxic rats. However, mean arterial pressure at the time of freezing was higher in control than in the hypoxic rats (P < 0.01).

Distribution of pH during O2 deprivation-induced respiratory depression. Figure 2 illustrates the fundamental finding of this study. The control brain stem image (Fig. 2, left), from a section taken at 12.7 mm caudal to the bregma, exhibited a relatively homogeneous pH, between 6.95 and 7.25. The hypoxic brain stem image, at a slightly more caudal level (12.8 mm caudal to the bregma, 1.5 mm rostral to the calamus scriptorius), on the other hand, demonstrates that the pH of most of the brain stem became more acidic, pH between 6.5 and 7.0, and heterogeneous during hypoxia (Fig. 2, right). Interestingly, pH was relatively preserved in those regions near the dorsal surface and also near the ventral surface. Structures in the vicinity of the ventral medullary surface extending from the pontomedullary border to the calamus scriptorius were consistently less acidic than other surrounding regions. The black regions of the images in Fig. 2 are regions that did not contain sufficient dye to obtain valid pH information, such as regions with high white matter content.

Figure 3 presents the frequency histogram distributions of both brain stem images from Fig. 2. The control pH was 7.12 ± 0.10 (SD). The hypoxic pH distribution was shifted toward the acid and was broader than the control, 6.75 ± 0.23 (SD).
Brain stem regional analyses of cerebral metabolites and pH$_i$ during O$_2$ deprivation-induced respiratory depression. Analysis of pH$_i$ by specific regions of interest obtained from control rats revealed mean baseline pH$_i$ between 7.10 and 7.25, as shown in Table 1. The mean pH$_i$ values from the hypoxic brain stems were between 0.23 and 0.41 units more acidic than the corresponding regions of the control brain stems. The acidification was statistically significant in the ventral portion of the nucleus gigantocellularis and the parapyramidal nucleus, midline neurons, and the spinal trigeminal nucleus but not in the nucleus ambiguus or nucleus tractus solitarii.

Lactate concentrations were generally higher than expected in the control brain stems, and PCr values were increased by about threefold in the hypoxic brain stems. Thus the PCr/ATP ratio was higher than expected in the control brain stems, and PCr values were slightly above expected levels. ATP concentrations were decreased by 75% and ATP by nearly 50% in the hypoxic brain regions. There were no significant regional differences in lactate, PCr, ATP, or PCr/ATP in either control or hypoxic brain stems.

**DISCUSSION**

In anesthetized animals, exposure to hypoxia results in typical changes in the pattern of breathing, characterized by an initial increase in frequency and amplitude, followed within minutes by a decrease in respiratory output (Fig. 1). In the studies reported here, we examined the effects of reduction in O$_2$ availability on regional metabolic changes within medulla oblongata at this time after hypoxia-induced respiratory depression had already occurred.

pH$_i$. Despite the importance of pH$_i$ in function of brain stem neurons, not much is known about its regional variability. The various techniques that can be used for determination of pH$_i$, such as 31P-nuclear magnetic resonance (30), microelectrodes (6), and optical probes, i.e., absorption (23), and fluorescent (1, 8) dyes, have relative advantages and disadvantages with respect to invasiveness, cost, ease of calibration, sensitivity, and spatial and temporal resolution (19). The development of the quantitative method for pH$_i$ determination by using the absorption properties of the vital dye neutral red (19) has progressed to the point where the method can be used to determine pH$_i$ reliably in intact rat brains (13, 20, 21) and in brain-slice preparations (21, 33).

Respiratory depression induced by hypoxia was associated with regional changes in pH$_i$. There was a general acidification of most brain stem regions of ~0.25 pH unit. This acidification is about two to three times that reported for whole brain stem (27). More interesting was the apparent preservation of pH$_i$ in hypoxic brain stem regions near the dorsal and ventral medullary surfaces (Fig. 2, right). These regions have been identified as having chemosensory function (7, 15)
and presumably correspond with the rostral chemosensitive area in cats (32). In rats, chemoreceptive elements were found to be located in a column in the ventrolateral medulla extending from the most rostral regions to the level of the hypoglossal rootlets (15). In addition, the ventral surface would be expected to have a hypoxia-induced acidification of the extracellular space (36, 37). Presumably, these regions can maintain pH, despite extracellular acidification, whereas other brain stem regions become more acidic intracellularly while maintaining pH.

Cerebral metabolites. We found differences in the lactate, PCR, and ATP concentrations of the normoxic rat brain stem compared with the usually cited cerebral cortex levels (3, 29). By comparison, we report higher lactate levels and lower ATP levels. Concentrations of cerebral metabolites are most commonly reported in millimolar. Because of the lyophilization and assay procedure, our data are reported as nanomoles per milligram dry weight. These data can be converted to millimolar by dividing the reported value by the factor 4, assuming the brain stem wet weight ratio is 75%, as it is in the midbrain (24). This means that the concentration of ATP in the normoxic brain stem was just <2 mM, whereas lactate was 5–6 mM. It is unlikely that these concentrations were due to poor tissue fixation because PCR concentrations were in the range of 5–6 mM, which are much higher than would be expected with poor preservation. These data are not far off from those we reported in a recent study of rat cerebral cortex, where we found ATP to be 8.9 nmol/g dry wt, PCR was 20.3 nmol/g dry wt, and lactate was 15.7 nmol/g dry wt (10). Nevertheless, the high PCR/ATP observed may be characteristic of brain stem metabolism.

Hypoxia induced an overall threefold increase in lactate, which is similar to that reported for rat cerebral cortex (2, 3, 27, 29) and greater than that reported for rat brain stem (27), where the increase in lactate was about double. Measurements of PCR and ATP, in the same medullary regions in which the pH and the lactate levels were determined, revealed significantly lower PCR and ATP concentrations in hypoxic rats than in control animals. In animals exposed to a steady-state level of hypoxic stress, at the time point when respiratory depression occurred, fall in PCR was more accentuated than the decrease in ATP. This suggests that the decline in ATP level is “buffered” and delayed by the operation of the creatine phosphate system. Because the concentration of PCR in the brain is greater than that of the adenine nucleotides and the equilibrium in PCR reaction is shifted toward ATP synthesis, the initial hydrolysis of PCR during O2 deprivation is not followed by a proportional decline in ATP concentration. However, as duration and severity of hypoxia increase, demonstrable decreases in cellular ATP concentrations can be measured. In these studies, when complete cessation of breathing activity occurred, ATP concentrations were one-half of those in control animals, whereas PCR concentrations were only 25% of expected values. The 75% fall in PCR levels was about three times that found for rat cerebral cortex (3, 12, 29). The more surprising finding was the 50% fall in ATP concentrations in the brain stem. Moderate hypoxia would not be expected to result in a fall in ATP (34) except where systemic blood pressure also falls, as in this study where systemic pressure dropped by one-third.

Our data are compatible with the suggestion that hypoxic depression of respiratory output is related to brain stem lactic acidosis produced by metabolic stress. We found that an increase in lactate levels in any of studied nuclei paralleled changes in pH, suggesting that reduction in O2 promoted production of lactic acid and intracellular acidosis. There are no data to suggest that mild intracellular acidosis, comparable to that reported here within brain stem nuclei, may cause membrane hyperpolarization. However, Neubauer et al. (28) demonstrated that in anesthetized animals prevention of brain acidosis by treatment with dichloroacetate, which inhibits production of lactic acid, abolishes the depression of breathing activity during progressive mild to moderate hypoxia. Furthermore, it has been shown that hypoxia is also associated with extracellular acidosis (36). It was reported that moderate increase in H+ concentration in extracellular fluid (pH 6.5) markedly reduces NMDA-receptor activation (11). Hence hypoxia-induced acidosis by various pathways may reduce cell activity.

In summary, systemic hypoxia in anesthetized, vagotomized, and mechanically ventilated rats, at the time point when respiratory depression occurs as the physiological response to systemic O2 deprivation, is associated with comparable decreases in pH, PCR, and ATP in brain stem nuclei involved in regulation of breathing activity. However, the relative contribution of each metabolic component on initiation of hypoxia-induced respiratory depression and the time course of metabolic events associated with the respiratory output changes, from the moment of exposure to the occurrence of apnea, need to be examined.

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REFERENCES


14. Haxhiu, M. A., E. Erokwu, E. van Lunteren, N. S. Cherni-

15. Issa, F. G., and J. E. Remmers.


18. Lahiri, S.

19. LaManna, J. C.


22. LaManna, J. C., M. A. Haxhiu, K. L. Kutina, and N. S. Cherniack.

23. LaManna, J. C., and K. A. McCracken.


30. Sick, T. J., T. S. Whittingham, and J. C. LaManna.

31. Sickjö, B. K.

32. Tombaugh, G. C., and R. M. Sapolsky.


