Association of Anemia with Reduced Central Respiratory Drive in the Piglet

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Central respiratory drive was studied in 13 piglets of both sexes varying in age from 19 to 67 days. The distal trachea was cannulated and the maximum rate of isometric inspiratory pressure change (dP/dt)\textsubscript{max} was measured at the airway. Curves were constructed relating this measurement to changes in arterial PCO\textsubscript{2} during carbon dioxide rebreathing. Data were obtained at intervals corresponding to stepwise reductions in central respiratory drive produced by added chloralose anaesthesia. Laryngeal reflex activation was achieved by electrical stimulation of the superior laryngeal nerves (SLN). This caused permanent respiratory arrest at a critical level of central respiratory depression expressed as the slope of the curve relating (dP/dt)\textsubscript{max} to arterial PCO\textsubscript{2}. Severely anemic piglets showed markedly decreased central respiratory drive at a given dose of anesthesia compared to controls. This was consistent with the observed greater sensitivity to laryngeal nerve stimulation in these animals. It is concluded that anemia may be associated with impaired functional maturation of central respiratory mechanisms and consequent susceptibility to laryngeal reflex apnea and asphyxial death. These observations may pertain to factors associated with the sudden infant death syndrome.

There is increasing evidence to support the notion that the sudden infant death syndrome is caused by respiratory arrest and asphyxia [1,2,3]. Hence, factors which may diminish central respiratory drive, and systems which may reflexly inhibit respiration assume potential importance in unraveling this problem. A mechanism which has aroused considerable interest is the laryngeal chemosensitive inhibitory reflex which has been studied in piglet and lamb models[4,5,6]. Introduction of water or cows' milk into the laryngeal area of piglets may lead to persistent apnea and asphyxial death [5,6]. Furthermore, anemia and growth retardation independently increase susceptibility to respiratory depression by the anaesthetic, chloralose, and the combination is additive [7,8]. A more satisfactory definition of respiratory drive in these conditions is needed, however.

Recent developments in respiratory physiology have distinguished between the motor output of the respiratory center and the central timing mechanisms which control the duration of inspiration [9,10,11]. A simple technique has been developed for assessing CO\textsubscript{2} responsiveness by measuring the maximum rate of isometric inspiratory pressure change at the airway, (dP/dt)\textsubscript{max} [12]. This is believed to represent the initial rate of development of force by the inspiratory muscles before this can be modified by mechanical loading, proprioceptive feedback mechanisms, or vagally mediated lung volume information [13]. Thus it may be used to quantify changes in the motor output of the respiratory center in response to ventilatory stimuli independent of pulmonary mechanics. This measurement may be combined

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with the rebreathing technique of Read and Reuben which uses the lung as a tonometer to achieve rapid equilibration between alveolar, mixed venous, and arterial carbon dioxide tensions [14]. Arterial carbon dioxide then rises linearly with time, independently of ventilation [15]. By pre-oxygenation and the use of 7 percent CO₂ in oxygen in the rebreathing circuit one eliminates the hypoxic drive to ventilation from the peripheral chemoreceptors. Thus the slope of the curve relating \(\frac{dP}{dt}\)max to arterial PCO₂ reflects central respiratory gain, or drive. The purpose of the present study was to evaluate more precisely the relationship between central respiratory drive and spontaneous anemia in the newborn piglet.

METHODS

Studies were carried out in 13 mixed breed piglets, varying in age from 19 to 67 days. They were divided into two groups according to initial hematocrit values. Severe iron deficiency anemia was present in those with the lowest hematocrits. Other features of the two groups are shown in Table 1. Except for obvious growth retardation, the most anemic group appeared healthy and active. The animals were initially anesthetized with intraperitoneal pentobarbital sodium (25 mg/kg) and studied in a supine position. The trachea was cannulated to permit respiratory measurements. Catheters were introduced into a jugular vein for infusion and a femoral artery for measurement of arterial blood pressure and heart rate. Arterial blood samples were obtained for measurement of blood gases and pH (Instrumentation Laboratories) and hematocrit (microhematocrit tubes, Clay Adams).

A rebreathing circuit (Fig. 1) was provided by a 300 ml Krogh spirometer; the total volume including tubing was kept to 500 ml to permit rapid equilibration of alveolar, mixed venous, and arterial CO₂ tensions. Ventilation was recorded on the spirometer and arterial carbon dioxide tension (Pₐ CO₂) measured at the commencement and at 2-minute intervals during each rebreathing run. At the tracheal cannula a low resistance one-way valve (Hans Rudolph pediatric) produced an almost imperceptible transient occlusion of inspiration. The valve had an opening pressure of less than 0.5 cm H₂O and its dead space was reduced to 6 ml by a teflon insert. Airway pressure was measured by a differential pressure transducer (Hewlett Packard 270) and differentiated by a conventional R-C circuit with a time constant of 0.4 ms. All signals were recorded on a Sanborn 4-channel polygraph.

The maximal rate of change of airway pressure, \(\frac{dP}{dt}\)max, was measured in cm H₂O/sec. Calibration was achieved by connecting a Harvard small animal respirator to a one-liter bottle. A pressure wave was produced whose maximum slope could be measured graphically. This signal was differentiated, producing another signal whose height represented the previously measured slope. This was recorded and used as the calibration for \(\frac{dP}{dt}\)max. The correlation coefficient between graphical and electronic measurements was 0.99 over the rate 4–50 cm H₂O/sec. Calibration of the differentiator circuit with a sine wave generator revealed a correlation coefficient of 0.99 between input and output voltages over a frequency range of 0.1 to 4 Hz.

After the initial preparation, arterial blood gases and hematocrit were measured

| TABLE 1 |
|-----------------|------------|-------|--------|--------|
| N               | Age, days | Wt (kg) | Hct (%) | Fe (µg/dl) |
| Control         | 8         | 38.6 ± 6.5 | 7.3 ± 1.1 | 19 ± 1.1 | 48.7 ± 9.7 |
| Anemic          | 5         | 41.2 ± 5.5 | 4.0 ± 0.8 | 6 ± 0.3 | 28.4 ± 2.9 |
| p               | N.S.      | < 0.01   | < 0.01  | < 0.05  |

Wt = total body weight. Hct = hematocrit. Fe = serum iron concentration (atomic absorption spectrometry).
ANEMIA AND APNEA IN THE PIGLET

while the piglet breathed room air. After 5 minutes of oxygen breathing, another arterial blood gas sample was obtained to ascertain that arterial oxygen tension was above the peripheral chemoreceptor threshold and to record the initial arterial PCO₂. The piglet was then switched into the rebreathing circuit, which had been flushed with 7 percent CO₂ in oxygen. Recording was performed at a speed of 2.5 mm/sec except that at one-minute intervals records of five breaths were obtained at 25 mm/sec to permit graphical verification of \((dP/dt)_{\text{max}}\). Rebreathing was continued for six minutes. Timed arterial blood gases were obtained at 2, 4, and 6 minutes and the piglet then returned to room air breathing.

Once cardiorespiratory parameters became stable on room air the piglets were subjected to superior laryngeal nerve (SLN) stimulation. A Grass Model S-4 stimulator with bipolar platinum electrodes was used to deliver pulses of 5 ms duration, 10v, and 10 Hz to one SLN and cardiorespiratory responses were measured.

Respiratory drive was progressively depressed by the administration of α-chloralose, intravenously, in 20 mg/kg increments. At each level of additional chloralose the measurements of central respiratory drive and responses to laryngeal stimulation were repeated. Recordings were analyzed by taking the mean \((dP/dt)_{\text{max}}\) of five breaths just prior to, and at two-minute intervals during rebreathing. Response curves of \((dP/dt)_{\text{max}}\) against arterial CO₂ tension were plotted graphically. Slope and intercept of \((dP/dt)_{\text{max}}\) on \(P_a\) CO₂ were calculated by linear regression analysis. Statistical significance was determined by the student \(t\)-test [16]. The difference was considered significant when the \(p\) value was less than 5 percent.

RESULTS

Ventilatory Responses to CO₂

Typical ventilatory responses to the rebreathing of 7 percent CO₂ in oxygen are shown in Fig. 2. The left panel shows arterial blood pressure (AP), tidal volume (VT),
airway pressure ($P_{AW}$), and its derivative $dP/dt$ immediately prior to rebreathing. The right panel shows the effects of six minutes of rebreathing. Arterial oxygen tension was held above the chemoreceptor threshold throughout the rebreathing run. Acute respiratory acidosis developed as $PCO_2$ increased. The maximal value of $dP/dt$ (average of 5 breaths), increased from 18 to 103 cm H$_2$O/sec, a 5.7-fold increase. This suggests that tidal volume increase is limited by pulmonary mechanics. Ventilation, the product of tidal volume and respiratory frequency, would be similarly limited by the impedance of the respiratory system.

Figure 3 shows the response to CO$_2$ rebreathing of the same piglet. Values for $(dP/dt)_{max}$ in cm H$_2$O/sec are plotted against $P_aCO_2$ in mm Hg. The curves were obtained after basal anesthesia with pentobarbital at 25 mg/kg, and following sequential addition of chloralose in 20 mg/kg increments. Since changes in airway resistance and lung volume have been shown to cause primarily changes in the intercept of the $(dP/dt)_{max}/CO_2$ curve with little effect on slope, we have chosen slope as the most appropriate single index of central respiratory drive (Fig. 3, right panel). Thus, decreasing slope is associated with progressive central respiratory depression. After receiving basal anesthesia and a total of 80 mg/kg of chloralose this particular piglet succumbed to supramaximal electrical stimulation of one superior laryngeal nerve. The slope of the $(dP/dt)_{max}/CO_2$ curve immediately preceding lethal reflex apnea was 0.32, compared with the initial value of 3.96 cmH$_2$O/sec/mm Hg.

**Effects of Anemia on Central Respiratory Drive**

The effects of anemia on central respiratory drive are shown in Fig. 4, expressed
ANEMIA AND APNEA IN THE PIGLET

FIG. 3. Curves relating $(dP/dt)_{\text{max}}$ to simultaneous measurements of arterial $CO_2$ tensions ($P_CO_2$) are shown in the left panel. Initial curve (closed circles) obtained under basal anesthesia. Successive curves obtained after giving chloralose in increments of 20 mg/kg. Calculated slope of each curve is shown in the right panel. Data for 80 mg/kg omitted from left panel for clarity. Wt = body weight. Hct = hematocrit (%).

as the slope of the $(dP/dt)_{\text{max}}/CO_2$ curve. The left panel shows the slopes at basal anesthesia with 25 mg/kg pentobarbital. The mean value for the control piglets was 1.71 (± .41 SE) cm H$_2$O/sec/mm Hg. This is compared with a mean value of 0.36 (± 0.9) cm H$_2$O/sec/mm Hg for the anemic piglets ($p < .05$). The center panel shows the effect of 20 mg/kg of additional chloralose. Control piglets had a slope of 1.81 (± 0.31) compared with 0.09 (± 0.02) for the anemic piglets ($p < .02$). After 40 mg/kg

FIG. 4. Mean values for slopes of curves obtained from two groups of piglets separated by hematocrit (Hct) values. In each, increments of chloralose reduce slope, and differences are significant at each level. Vertical brackets indicate SEM.
of additional chloralose, control piglets had a slope of 0.59 (± 0.15), while that for the anemic piglets was 0.05 (± 0.04) ($p < .05$). Thus for an equivalent dose of anesthetic, respiratory drive is significantly less in the anemic animals than in controls.

**Influence of Decreased Central Respiratory Drive on the Laryngeal Reflex Response**

The relative gain of the laryngeal inhibitory reflex was assessed by comparing the number of animals in each group which exhibited permanent apnea during sustained electrical stimulation of a superior laryngeal nerve. This test was repeated following each incremental dose of chloralose and the results are illustrated in Fig. 5. None manifested permanent apnea in the basal state, or following 20 mg/kg of chloralose. After 60 mg/kg, only one of eight animals (13 percent) with the higher hematocrits (19 ± 3 SE percent) died, whereas 80 percent of those with mean hematocrits of 6 (± 0.3 SE) percent developed permanent apnea. All in this group died during SLN stimulation after 80 mg/kg of chloralose, while 25 percent of those with the higher hematocrit values survived after 100 mg/kg. Thus, increasing sensitivity to laryngeal apnea may be ascribed to a progressive reduction in central drive as defined by the $(dP/dt)_{\text{max}}$ slope (Fig. 3, right panel). This relationship was also present in the group with lower hematocrit values where sharply reduced central drive was associated with a higher incidence of permanent apnea during SLN stimulation.

**DISCUSSION**

Recent studies from our laboratory have shown that under conditions of respiratory depression the laryngeal chemosensitive system is capable of producing permanent apnea [6,7]. The relative gain of the laryngeal inhibitory reflex can be estimated by comparing the amount of additional chloralose required to elicit lethal apnea in animals during superior laryngeal nerve (SLN) stimulation with the amount required when the laryngeal reflex is not subjected to stimulation [6]. Furthermore, using the same technique it can be shown that anemia decreases respiratory drive. Anemic

![FIG. 5. Appearance of permanent apnea during sustained electrical stimulation of one superior laryngeal nerve. Group with lower mean hematocrit (Hct) showed greater susceptibility at all levels of added chloralose above 20 mg/kg.](image_url)
piglets require less chloralose to induce permanent apnea than do non-anemic controls [8]. However, this analysis provides only an imprecise estimate of respiratory drive. In the present study we re-evaluated the relationship of the laryngeal chemoreflex and anemia using a more objective technique, the \((dP/dt)_{max}\) response to the rebreathing of \(\text{CO}_2\) in oxygen. While chloralose was used to produce respiratory depression as before, this approach permitted quantification of the effect on central respiratory drive. By maintaining arterial \(\text{PO}_2\) above the peripheral chemoreceptor threshold throughout the rebreathing run, one selectively stimulates the central medullary chemoreceptor with \(\text{CO}_2\). The measurement of \((dP/dt)_{max}\) reflects phrenic motoneurone discharge, independent of pulmonary mechanics [12]. Finally, supramaximal electrical stimulation of one superior laryngeal nerve causes central inhibition of respiration independent of the state of the laryngeal chemoreceptors. The techniques employed in the present study therefore permit a more direct and quantitative assessment of the interrelationships of central respiratory drive and the laryngeal chemoreflex.

We have found that a critical level of central respiratory depression is associated with permanent apnea during SLN stimulation and consequent asphyxial death. This level corresponds to virtual loss of \(\text{CO}_2\) responsiveness (Fig. 3), even though spontaneous ventilation may be adequate to maintain \(P_a \text{CO}_2\) levels within the normal range. When ventilation is interrupted by the laryngeal inhibitory reflex the resultant hypercarbia and hypoxemia are insufficient stimuli to restore respiratory movements. As we have shown in an earlier study, susceptibility to laryngeal induced apnea decreases with increasing age [4], implying that the maturing central nervous system becomes more autonomous and less influenced by inhibitory information from the periphery.

We have previously identified the combination of severe anemia and growth retardation as reducing the dose of chloralose which produced respiratory arrest in piglets [8]. The current study confirms these findings. In severely anemic piglets central respiratory drive under conditions of basal anesthesia was approximately 20 percent of control piglets (Fig. 4). This relationship persisted with the administration of additional chloralose. Moreover, anemic piglets were more susceptible to respiratory arrest at reduced doses of chloralose compared to controls (Fig. 5). Other effects of anemia, such as decreased \(O_2\) delivery to tissues during apnea may be contributory, but a significant influence on central respiratory drive is clear from these observations. This effect seems to involve a critical threshold phenomenon, rather than a graded response. Although the severely anemic piglets showed decreased respiratory drive, there was no clear correlation between hematoctrit values and \((dP/dt)_{max}\) in individual piglets with higher hematoctrit levels. To what extent decreased hemoglobin levels alone accounted for these observations as opposed to other effects associated with anemia warrants further investigation. We may tentatively consider anemia as one example of a neonatal insult leading to diminished potency of central respiratory mechanisms, hence, an enhanced potential for inhibition by stimuli which normally modify respiratory activity.

The results of the present study may have relevance to the clinical problem of the sudden infant death syndrome. The cause of death in SIDS is uncertain but increasing evidence has developed to support the view that respiratory arrest and asphyxial death is a likely sequence [1,2,3]. The laryngeal inhibitory reflex may be one contributory factor, capable of eliciting asphyxial death under conditions associated with diminished central respiratory drive. Anemia may represent (or reflect) one set of circumstances which reduces central drive, as suggested by the
piglet model. It is of interest that the clustering of SIDS deaths in the 2–4 month age range coincides with the nadir of physiologic anemia. Recently it has been shown that infants of anemic mothers are more likely to be SIDS victims [17]. There are presently no data on the hemograms of the victims themselves. It is possible that a number of deleterious influences in the perinatal period, including anemia, may predispose to delayed maturation of central respiratory mechanisms and consequent susceptibility to permanent apnea.

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