Myelination of the Human Vagus Nerve from 24 Weeks Postconceptional Age to Adolescence

PAUL N. SACHIS, M.Sc., DAWNA L. ARMSTRONG, M.D., F.R.C.P.(C), LAURENCE E. BECKER, M.D., F.R.C.P.(C), AND A. CHARLES BRYAN, M.B., B.S., PH.D., F.R.C.P.(C)

Abstract. Significant changes in respiratory reflexes occur with maturation. The vagus nerve, the pathway for the Hering-Breuer and irritant-receptor reflexes, was studied quantitatively in 33 infants and 5 adolescents. In the infants, total myelinated vagus fibers increased linearly (r = +0.682, p < 0.001) with postconceptional age (PCA), and by 40 weeks after conception, total counts were comparable to those of adolescent group. Counts of total myelinated vagus fibers in 16 term infants (≥41 weeks PCA) were comparable to those in the adolescent group (p < 0.40), whereas 17 preterm infants (≤38 weeks PCA) showed significantly fewer total myelinated vagus fibers than term or adolescent groups (p < 0.061). Smaller-diameter (∼2 µm) myelinated vagus fibers depended upon PCA in the preterm group (p < 0.005), but were independent of PCA in the term group (p < 0.5). Preterm infants have a higher percentage of small to total myelinated vagus fibers than term infants (p < 0.1).

Key Words: Apnea, Hering-Breuer reflex, Irritant-receptor reflex, Morphometry, Myelinated vagus fibers, Respiratory reflexes, Vagus nerve.

“Deform’d, unfinish’d, sent before my time
Into this breathing world scarce half made up.”
(Shakespeare, Richard III. Act 1, Scene 1)

INTRODUCTION

Understanding the development and maturation of the nervous system involved in respiratory control is important to neonatologists. Premature infants are subject to periods of apnea and have abnormal irritant reflexes (1, 2). In the older infant, sudden infant death syndrome (SIDS), a common cause of death in the first year of life, is considered to be related to aberrant neural respiratory control (3, 4). The neural control of respiration is a balance between facilitative and inhibitory inputs, but in the preterm infant, an inhibitory bias exists. Vagal facilitative reflexes are weak. Marlot and Duron (5) showed that the deflation reflex in kittens was weak or absent during the first three weeks of life. A vagal inhibitory reflex—the Hering-Breuer stretch reflex—is strong in both newborn animals (6) and infants (7). Marlot (8) showed that vagal reflex differences have
their basis in the structural immaturity of the newborn nervous system by relating the maturation of the vagus nerve to that of respiratory reflexes in kittens. To test the hypothesis that irregularities of neonatal pulmonary physiology in man reflect an immature nervous system, we studied the myelination of the cervical vagus nerve in preterm and term infants and in adolescents.

SUBJECTS AND METHODS

Vagus nerves were removed at autopsy from 33 infants who died at less than 1 yr of age. For comparison we studied vagus nerves obtained at autopsy from 5 adolescents.

Infants were studied in two groups according to postconceptional age (PCA), defined as gestation plus postnatal age at death. Group A consisted of 17 infants with a PCA of \( \leq 38 \) weeks (wk) (range, 24–38 wk; mean, 30 wk) and group B of 16 infants with a PCA of \( \geq 41 \) wk (range, 41–88 wk; mean, 57 wk). The 5 adolescents (group C) were aged 12–22 years: their deaths resulted from trauma (fire, drowning, homicide, suicide, motor vehicle accidents). The infants in groups A and B died of respiratory distress syndrome, perinatal asphyxia, acute infection, noncyanotic congenital heart disease, or trauma. We excluded infants who had experienced prolonged chemotherapy, nutritional problems, chronic uremia, cyanotic congenital heart disease, and child abuse.

From each subject, we removed 2 cm of the left cervical vagus nerve proximal to the origin of the left recurrent laryngeal nerve, within 24 hours (h) of death. Specimens were fixed by immersion at 4°C for 12 h in 3% glutaraldehyde dissolved in 0.1 M sodium phosphate buffer (pH 7.4), and postfixed by immersion at 4°C for 2 h in 2% osmium tetroxide dissolved in 0.1 M sodium phosphate buffer (pH 7.4). After a 30-minutes (min) wash in the same buffer, each nerve was dehydrated in a series of increasingly concentrated ethyl-alcohol solutions, infiltrated with propylene oxide, embedded in Epon with Araldite (9), sliced into 1-\( \mu \)m transverse sections, and stained with 1% toluidine blue (10).

The Dyck et al. method (11) was used in quantifying and measuring the diameters of myelinated fibers. A low-power photomicrograph of the entire cross-section of each nerve was used at a final magnification of \( \times 1000 \). The perimeter of the endoneurium of each fascicle within the nerve cross-section was traced with the digitizer attachment of a programmed calculator (Hewlett-Packard 9810). Then, the sum of the areas of the fascicles within each nerve was divided by the square of the entire nerve cross-section to obtain the transverse fascicular area (TFA).

We counted the myelinated vagus fibers in alternate fields (photomicrographs of final magnification \( \times 1000 \)) within the TFA, including fibers touching the left and lower boundaries of the field and excluding those touching the right and upper boundaries. The diameters of myelinated vagus fibers (axon + outer rim of myelin) were measured with a Zeiss TGZ3 particle-size analyzer; we approximated the lightspot to the center of the circular fibers, the central area of the scalloped fibers, the minor axis of the elliptical fibers, and the base of triangular fibers. The very irregular fibers with no defined diameter (less than 5% of total count) were not included. The analyzer was set in regular range, in exponential mode, and for frequency distribution.

The computer calculated estimated density of myelinated vagus fibers per mm\(^2\) of TFA and per cross-section of vagus nerve, and plotted size-distribution histograms (Dyck PJ, O’Brien P, Augustine G. Software for evaluating fascicular area and number of fibers from pictures of transverse sections of nerves. Rochester, Minnesota: Mayo Clinic, 1976). We then plotted counts of total myelinated vagus fibers for infants by PCA.
STATISTICAL METHODS

Pearson product-moment correlation coefficients (r) and crude regression coefficients (b) were calculated with a two-tailed Student’s t test with (n – 2) degrees of freedom (df) on counts of myelinated vagus fibers in relation to PCA. Significantly associated variables were shown using least-squares regression lines. A two-tailed variance-ratio (Snedecor’s F-) test evaluated homogeneity of sample variances (s²), or variances of the estimates (s²ₓ), with (n – 1) and (n – 2) df, respectively. In the homovariant cases, the difference between sample means were tested using a two-tailed Student’s t test with (n₁ + n₂ – 2) df for independent samples. The difference between crude regression coefficients (slopes) was tested using a two-tailed Student’s t test with (n₁ + n₂ – 4) df’. A significant difference between sample variances or variances of the estimates (heterogeneity) necessitated evaluation of the difference between sample means of slopes using a two-tailed weighted t test with computed df” for independent samples (12). Correlation coefficients, slopes (and their differences), variance ratios, variances of the estimates, and the difference between sample means were considered significant if p < 0.05. Data were expressed in terms of mean ± standard deviations (SD).

RESULTS

Total counts of myelinated vagus fibers for groups A and B were plotted as a function of PCA (Fig. 1). Myelinated vagus fibers increased rapidly and by about 40 weeks PCA, counts of total myelinated vagus fibers were comparable to those in the adolescent group which ranged from 27,000–55,000 fibers (Table 1). Total and small-diameter (≤2 μm) myelinated vagus fibers in the 17 preterm infants (group A) showed significant positive-imperfect associations with PCA (r = +0.809, df = 15, p < 0.001 and r = +0.669, df = 15, p < 0.01, respectively). However, in the 16 other infants (group B), total and small-diameter myelinated vagus fibers showed non-significant positive- and inverse-imperfect association with PCA, (r = +0.034, df = 14, p < 0.1 and r = −0.220, df = 14, p > 0.1, respectively).

The difference between slopes of total myelinated vagus fibers on PCA for both group A and group B was significantly different (t’ = 4.400, df’ = 25, p < 0.001), as were slopes of small-diameter myelinated vagus fibers on PCA for both groups (t’ = 5.105, df’ = 29, p < 0.001). The slopes of small-diameter myelinated vagus fibers on PCA was significant in the preterm group (p < 0.005), but not in the term group (p < 0.5). Thus counts of small-diameter myelinated vagus fibers were dependent upon PCA in the preterm infants, but were independent of PCA in the term infants.

Because of the significant (t’ = 6.459, df’ = 19, p < 0.001) mean PCA differences between groups A and B, total myelinated vagus fibers were significantly fewer in group A than in group B (t = 7.176, df = 31, p < 0.001). Group A infants showed significantly fewer myelinated vagus fibers than group C adolescents (t = 5.745, df = 20, p < 0.001). Group B infants exhibited counts of myelinated vagus fibers comparable to those of group C (t = 0.928, df = 19, p < 0.40). Mean small-diameter (≤2 μm) myelinated vagus fibers did not differ significantly among groups A and B: t = 1.758, df = 31, p < 0.1; groups A and C: t = 0.644, df = 20, p < 0.6; and groups B and C: t = 0.561, df = 19, p < 0.6 (Table 1).
DISCUSSION

In cross-sections of the vagus nerve, there is a gradual transition from unmyelinated to myelinated nerve fibers. With toluidine blue-stained myelinated vagus fibers for light microscopy, the myelin sheath is visible only if there is an adequate quantity of myelin. We acknowledge that by electron microscopic resolution additional myelinated fibers could be identified.

This enumeration of total myelinated vagus fibers demonstrated a linear increase in total numbers of myelinated fibers from 24–40 wk PCA. The nerve maturation observed at various PCAs supports our hypothesis that respiratory neurons in the preterm infant are immature. Aberrant respiratory reflexes in prematurity may relate to the decreased numbers of myelinated vagus fibers in our study. For example, Bodegard et al. (13) reported that the Hering-Breuer inspiration-inhibiting reflex, mediated by large myelinated vagus fibers, was weak in infants of 32 wk gestation, but increased in strength until 38 wk gestation. Beyond this gestational age, this vagal reflex became weaker. The physiological observations made by Olinsky et al. (14) are contradictory. Using an indirect technique for measuring this inflation reflex, those authors concluded that the vagal reflex was stronger in the preterm infant. The role of the vagus in
<table>
<thead>
<tr>
<th>Age</th>
<th>Total Specimens (n)</th>
<th>Total MVF (n)</th>
<th>Small MVF (≤2 μm diameter) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>30 ± 5 weeks PCA</td>
<td>17</td>
<td>11,433 ± 7,366</td>
</tr>
<tr>
<td>Group B</td>
<td>57 ± 16 weeks PCA</td>
<td>16</td>
<td>30,933 ± 8,239</td>
</tr>
<tr>
<td>Group C</td>
<td>12–22 years</td>
<td>5</td>
<td>35,103 ± 10,526</td>
</tr>
</tbody>
</table>

TABLE 1
Number of Myelinated Vagus Fibers (MVF) and Small MVF (Means ± SD) in Infant Groups A and B and Adolescent Group C
patterning of respiration is also controversial. In the model of Clarke and von Euler (15), rising vagal stretch-receptor discharge with lung inflation has a causal role in terminating central inspiratory activity. This model was derived from anesthetized animals and its applicability to human beings and particularly newborn infants, is questionable. However, if this model is applicable, the immaturity of the vagus may in part be responsible for the unstable breathing patterns so common in preterm infants.

The irritant-receptor reflex is mediated by small myelinated fibers. Infants of less than 35 wk gestation do not have a pulmonary irritant reflex (1). They respond to stimulation of the bronchial mucosa with apnea. After 35 wk, the same stimulation produces the adult response of cough and arousal. This correlates with our observation that the numbers of small myelinated fibers increased with PCA in the preterm infants.

These small-diameter fibers are of further interest to us because we have found that in some SIDS victims the numbers of small-diameter myelinated vagus fibers were comparable to those found in vagi obtained from preterm infants (16). A paucity of small-diameter myelinated vagus fibers was also found in a child with congenital central hypoventilation syndrome (Ondine’s curse) (17). In these lethal conditions immaturity of the vagus nerve may be important.

ACKNOWLEDGMENTS

We gratefully acknowledge receipt of the software for morphometric evaluation of peripheral nerve from Dr. Peter James Dyck, Mayo Clinic. We are grateful to Dr. M. J. Ball, Department of Pathology, University of Western Ontario, London, Canada for use of the Zeiss TGZ3 particle-size analyzer. We acknowledge the assistance of the Departments of Medical Publications and Visual Education, Research Division, The Hospital for Sick Children, Toronto, and Janice Edwards, Baylor College of Medicine, for preparing the manuscript.

REFERENCES

(Received: September 1, 1981/Accepted December 22, 1981)
MS81-50R