TITLE: Physiology of Esophageal Sensorimotor Malfunctions in Neonatal Neurological illness

SHORT TITLE: Neonatal Esophageal Dysmotility Mechanisms

AUTHORS:
1. Sudarshan R. Jadcherla¹, ²
2. Chin Yee Chan²
3. Rebecca Moore²
4. Soledad Fernandez³
5. Reza Shaker⁴

AFFILIATIONS:
1. Sections of Neonatology, Pediatric Gastroenterology and Nutrition, The Neonatal and Infant Feeding Disorders Program, Center for Perinatal Research, Department of Pediatrics, The Ohio State University College of Medicine, The Research Institute at Nationwide Children’s Hospital, Columbus, OH.
2. The Neonatal and Infant Feeding Disorders Program, The Research Institute at Nationwide Children’s Hospital, Columbus, OH.
3. Center for Biostatistics, The Ohio State University College of Medicine, Columbus, OH.
4. Division of Gastroenterology, Department of Internal Medicine, Medical College of Wisconsin, Milwaukee, WI, USA.


Copyright © 2013 by the American Physiological Society.
ADDRESS FOR CORRESPONDENCE:
Sudarshan R. Jadcherla, MD, FRCPI, DCH, AGAF
Professor of Pediatrics, The Ohio State University College of Medicine
Section of Neonatology, Pediatric Gastroenterology and Nutrition,
The Research Institute at Nationwide Children's Hospital
700 Children's Drive, Columbus, OH 43205, USA.
Phone: 614-722-5155
Facsimile: 614-722-4541
Email: sudarshan.jadcherla@nationwidechildrens.org

LOCATION OF WORK: Nationwide Children's Hospital, Columbus, OH

ABBREVIATIONS:
EDR  esophago-deglutition response
GER  gastroesophageal reflux
LES  lower esophageal sphincter
SP   secondary peristalsis
UES  upper esophageal sphincter
ABSTRACT

**Aims:** To define the sensorimotor characteristics of aero-digestive reflexes evoked upon mid-esophageal provocations in neuropathology infants. **Methods:** Provocative esophageal motility testing was performed in 20 neuropathology infants and 10 controls at 42.3±0.6 and 38.9±0.9 wk postmenstrual age. Data from 1,073 infusions were examined for the sensory thresholds, response frequencies, response magnitude of upper esophageal sphincter (UES) contractile reflexes, lower esophageal sphincter (LES) relaxation reflexes, and peristaltic reflexes using mixed statistical models. **Results:** Threshold volumes for air and liquid in neuropathology and control infants were similar for all reflexes. Graded air and liquid volume-dependent UES contractile reflex, LES relaxation reflex, and peristaltic reflex frequencies recruitment were present in neuropathology and control subjects for the media (P<0.0001) and the reflexes (P<0.0001). In neuropathology infants (vs. controls): UES contractile magnitude is higher (P<0.0001); LES relaxation reflex occurred earlier (P=0.008); LES nadir duration lasted longer (P=0.006); secondary peristalsis is the chief method of esophageal clearance (P<0.0001); pharyngeal swallows and deglutition apneas are less frequent (P=0.001); proximal, mid-esophageal waveform magnitudes and duration are exaggerated (P<0.008). UES contractile reflex was longer with liquid than air in both groups (P=0.03). **Conclusions:** 1) Perception to mid-esophageal provocation remains preserved in neuropathology neonates. 2) Sustained and exaggerated myogenic response from afferent activation is evident by: a) increased excitatory efferent outputs to the UES and esophageal body, b) increased inhibitory efferent outputs to the LES. 3) Dysfunctional regulation of pharyngeal swallowing and infrequent deglutition responses
indicate the possibility of impaired descending modulation and central malfunctions of brainstem and vagal nuclei.

**KEY WORDS:** dysphagia, gastroesophageal reflux, esophagus, asphyxia, intracranial hemorrhage
INTRODUCTION

Neonatal neurological consequences are important antecedents for aero-digestive and developmental morbidities (3, 15, 16). Specifically, intracranial hemorrhage and perinatal asphyxia constitute the most frequent neonatal neurological illness, with an estimated risk of developmental disability in 25-50% and cerebral palsy in 25% (30, 31). Clinical prototype of aero-digestive concerns include excessive pooling of oral secretions, inability to swallow secretions, aspiration pneumonia, dysphagia, gastro-esophageal reflux (GER) disease, and life threatening choking events (26, 29). Despite the prevalence of such problems, the mechanisms of malfunction of aero-digestive reflexes are unclear.

The integrity, coordination and functions of upper esophageal sphincter (UES), lower esophageal sphincter (LES), and esophageal body are essential to maintain swallowing, esophageal peristalsis, and airway protection (2, 23, 24, 28). During health, the sequence of aero-digestive reflexes evoked upon esophageal provocation facilitates peristaltic clearance and prevents the stimulus spread into the vicinity of airway (18).

We have recently developed and validated multimodal esophageal provocative methods to examine the functions of vagal afferent and efferent pathways by defining sensorimotor characteristics of esophageal body, UES contractile reflexes, and LES relaxation reflexes in healthy human neonates (7, 8, 11, 12, 24). Specifically, mechanosensitive, osmosensitive and chemosensitive provocation of the mid-esophagus was undertaken using air, water (pH 7.0) and apple juice (pH 3.7) respectively, and the effects on upstream (UES) and downstream (LES) reflexes and
esophageal peristalsis were evaluated. By using these media in graded incremental volumes, we defined the responses to varying physical and chemical characteristics of stimulus, media and volume (12, 25). During GER events, physical and chemical characteristics of the refluxate provoke the esophagus. By using different media to provoke the esophagus, we tested the individual effects of mechanosensitive, osmosensitive and chemosensitive stimulation. The effect of esophageal provocation in neonatal neurological illness is unclear; and therefore contributed to the rationale for this study.

The rationale for the current study has the following scientific basis: We have previously validated and tested methods to define esophageal provocation induced vago-vagal reflexes in neonates, such as those induced by esophageal mechanosensitive and chemosensitive stimulation during maturation. Similar work has been accomplished in human adults and animal models (6, 20). The latter work clearly establishes the role of the vagus nucleus and vagal innervations and networks modulating the esophageal and sphincteric sensorimotor activity and the role of brain stem. Provocative manometry model of investigating vago-vagal reflexes in the current pathologic setting of neonatal neurological illness has not been studied before. Therefore, we undertook the aims to test the hypothesis that infants with neonatal neurological illness have malfunctions of vago-vagal esophageal and sphincteric reflexes. Specifically, we defined and differentiated the sensorimotor characteristics of esophageal mechanosensitive-, osmosensitive- and, chemosensitive- stimulation induced peristaltic reflexes, UES contractile reflexes, and LES relaxation reflexes. We also examined the esophageal sensitivity by measuring sensory stimulus threshold
volume, response latency, and response duration. We examined the motor characteristics by defining the reflex recruitment frequency, stimulus-response relationships, and response magnitude.

**SUBJECTS AND METHODS**

*Participants*

Twenty neurologically impaired neonates (6 males; 36.0 ± 1.3 wk gestational age, GA) were evaluated at 42.3 ± 0.6 wk postmenstrual age, PMA, when they had oral feeding problems and therefore receiving nasogastric feedings. Generally, by full term PMA, most healthy infants are on full oral feeds (16) regardless of GA at birth. Because full term born healthy infants are a difficult group to recruit and are generally discharged within 48 hours; to compare data, in this study 10 healthy control infants that had independent oral feeding skills (4 male; 32.7 ± 1.5 wk GA) were studied at 38.9 ± 0.9 wk PMA. The controls were admitted initially for transitional respiratory distress and received routine neonatal care. These subjects were feeding and thriving appropriately during the hospital course, and did not have any neuropathology or other gastrointestinal problems either at admission or at evaluation. All subjects were evaluated by the principal investigator (SRJ) and the attending neonatologist and were deemed appropriate at study. None of the subjects had a presumed or proven clinical diagnosis of GER, and none were receiving prokinetics, acid suppressive therapy or xanthines at the time of study or discharge. Infants with congenital gastrointestinal abnormalities, birth defects, chromosomal disorders, and congenital brain abnormalities were excluded.
The study procedures were approved by the ethics committee at the Institutional Research Review Board at the Nationwide Children’s Hospital Research Institute, Columbus, Ohio, USA. The study protocol conforms to the standards set by the latest revision of the Declaration of Helsinki, and the methods and procedures were approved by Nationwide Children’s Hospital Research Institute constituted Institutional Research Review Board as well as in accordance with the health insurance portability and accountably act. Written informed consents were obtained from parents prior to study.

**Manometry methods**

The use of esophageal manometry methods and multimodal provocation techniques in neonates was described by our group (8, 9, 11). Briefly, the catheter assembly (Dentsleeve International, Mui Scientific, Ontario, Canada) was connected to the pneumohydraulic micromanometric water perfusion system via the resistors, pressure transducers (TNF-R disposable pressure transducers) and amplifiers (Solar modules, Solar 2, MMS medical instruments, Dover, NH, USA). The esophageal manometry catheter assembly with dual sleeves (recording from UES and LES) and 4 side-ports recording from pharynx, proximal-, middle- and distal- esophageal loci and a terminal gastric recording port was used. The catheter also includes a dedicated sensorimotor infusion channel to provide esophageal stimulus. The water perfusion rate was 0.02 ml/min/port for esophageal ports, 0.01 ml/min/port for the pharyngeal port, and 0.04 ml/min/port for the sleeves. The catheter was passed nasally in the unsedated supine lying neonate, and all studies were done in the same manner, with the transducers at the level of the subject’s esophagus (mid axillary line). Vital signs were monitored for safety during the manometry study.
**Manometric experimental protocol**

Continuous data acquisition and analysis were performed based on manometric waveform characteristics (8, 9, 11). Middle esophageal provocations with air, water, and apple juice (pH=3.70) were performed to test the effects of mechanosensitive, osmosensitive, and chemosensitive stimulation respectively. During catheter placement and pull through, the UES and LES sleeves were positioned such that they straddled the UES and LES high pressure zones, respectively and were identified by the presence of a consistent increase in pressure greater than 5.0 mmHg above the baseline for at least 15 sec, in addition to the changes in pressure with respiration. After neonates were allowed to adapt for about 15 minutes, we evaluated responses to mid-esophageal provocation.

**Manometry Data Analysis**

The manometric waveforms related to UES, LES and esophageal reflex characteristics were analyzed as defined before (7-12, 24). Briefly, *Esophago-Deglutition Response (EDR)* was defined as a deglutition response to esophageal stimulation which begins with onset of the pharyngeal waveform associated with UES relaxation and propagates into the proximal, middle, and distal esophageal segments and is accompanied by LES relaxation. *Secondary Peristalsis (SP)* occurs in response to sensorimotor provocation, and is defined as the propagation of waveforms distally from the proximal, middle, and distal esophageal segments in the absence of pharyngeal waveform and UES relaxation. The onset of proximal esophageal upstroke from the stimulus onset defines the response latency for secondary peristalsis. *Response onset to peristaltic reflex* was taken from onset of the stimulus to the onset of esophago-deglutition response or
secondary peristalsis. Deglutition apnea and changes in respiratory rhythm related to peristaltic reflexes were defined using respiratory inductance plethysmography.

**Analysis related to UES and LES characteristics**

*Resting UES Pressure* was measured as an average of five UES pressure measurements at end of expiration observed prior to stimulus. *Response latency to UES contractile reflex* is defined as the time taken from the onset of stimulus for an increase in UES pressure of at least 4 mmHg above baseline. *Maximum UES Contraction Pressure* was taken at the maximum pressure reached after onset of UES contraction. *UES contractile magnitude* is defined as the pressure differences between the Maximum UES Contraction Pressure and the Resting UES pressure. *UES contractile reflex Duration* is measured from the onset of UES contraction, to the peak of UES contractile reflex. *Resting LES Pressure* was measured as an average of five LES pressure measurements observed prior to stimulus. All pressures were taken at the end of expiration and in relation to gastric pressure.

**Statistical Methods**

Subject characteristics, manometric measurements, and outcome variables were compared between the neuropathology and control groups. Multinomial mixed models and linear mixed models with compound symmetry matrix were used to analyze the repeated-measures data. Statistical tests were adjusted for multiple comparisons using the Tukey-Kramer method. These models were fit using PROC GENMOD for categorical responses and PROC MIXED for continuous responses in SAS (SAS v.9.2 Institute Inc., Cary, NC, USA). To evaluate the effect of graded volume and media on the recruitment of reflexes, we used logistic regression models and compared between
the groups (Table 2). Descriptive data are reported as least square means ± SEM, percentages or as range unless stated otherwise.

RESULTS

I. Subject and Disease Characteristics

Ten control infants were studied at 38.9 ± 0.9 wk PMA when they had independent oral feeding skills, contrasting 20 neuropathology infants that were dysphagic and nasogastric feeding tube-dependent at evaluation, 42.3 ± 0.6 wk PMA. In the neuropathology vs. control groups respectively, the median (27) APGAR scores at 1-min were 2 (0-9) vs. 6 (2-8) (P=0.04); and at 5-min were 4 (0-9) vs. 8 (4-9) (P=0.03). At evaluation for the current study, the study group composition was heterogeneous and primarily had the following brain insults including perinatal asphyxia (N=12), intra-cranial hemorrhage and ventriculomegaly (N=6), neonatal encephalopathy and seizures (N=2). During the admission and prior to our evaluation of these dysphagic infants, other associated specific recognizable brain insults in some of them included severe intra-ventricular hemorrhage (grades 3 and 4) and post-hemorrhagic hydrocephalus (N=4) and meningitis (N=2).

II. Effects of Esophageal Provocation on Upstream and Downstream Motility

A total of 357 esophageal infusions (graded volumes of 143 air, 109 water and 105 apple juice) were given in the control group and a total of 716 esophageal infusions (289 air, 219 water, and 208 apple juice) were given in the neuropathology group.
Esophageal provocation induced UES contractile reflex, LES relaxation reflex and peristaltic reflexes (Figure 1) were analyzed for sensory- motor characteristics.

**a) Sensorimotor characteristics of UES contractile reflex**

Comparing neuropathology vs. controls, for UES contractile reflex characteristics, the threshold volumes to evoke UES contractile reflex were similar, response latencies (25) were similar ($3.5 \pm 0.3$ vs. $3.3 \pm 0.2$, $P=0.6$), the response durations (25) were similar ($2.8 \pm 0.2$ vs. $3.0 \pm 0.2$, $P=0.1$), and the frequency recruitment were also similar ($49.9\%$ vs. $48.8\%$, $P=0.7$).

*Significantly*, the neuropathology group had higher resting UES pressures, greater maximal UES contractile pressures, and greater change in UES contractile magnitudes (Figure 2).

**b) Sensorimotor characteristics of LES relaxation reflex**

In neuropathology vs. controls, for LES relaxation reflex characteristics, the threshold volumes were similar, response latencies (seconds) were similar ($3.8 \pm 0.2$ vs. $3.5 \pm 0.2$, $P=0.3$), frequency recruitment of LES relaxation reflex was similar ($47.0\%$ vs. $50.4\%$, $P=0.3$).

*Significantly*, the neuropathology group had quicker LES relaxation and for a prolonged nadir duration compared to the control group (Figure 3). No other significant differences between the neuropathology and control groups were noted with respect to resting LES pressures ($17.4 \pm 0.9$ mmHg vs. $18.3 \pm 1.2$ mmHg, $P=0.5$), LES nadir
pressures (2.0 ± 0.6 mmHg vs. 3.7 ± 0.8 mmHg, P=0.09), or the LES relaxation magnitudes (22.3 ± 0.8 mmHg vs. 21.4 ± 1.1 mmHg, P=0.5).

To further clarify if the LES relaxation characteristics were dependent on the type of peristaltic reflex, i.e., during deglutition or secondary peristalsis, individual comparisons were made (Table 1a and 1b). Specifically, the frequency recruitment, response latency and response duration, and magnitude of LES relaxation were similar with esophago-deglutition response between neurologically impaired vs. controls. On the other hand, LES relaxation characteristics during secondary peristalsis were similarly distributed except for the isolated findings of LES relaxation period was shorter and nadir duration longer in neurologically impaired subjects vs. controls.

c) Sensorimotor characteristics of Peristaltic reflex

Mean threshold volumes to evoke peristaltic reflexes were similar between the groups. \textit{Significantly}, however, the mean response latencies (seconds) to onset of peristaltic reflexes were shorter in the neuropathology group compared to the control group (3.7 ± 0.1 vs. 4.5 ± 0.2, P<0.0001). In addition, the characteristics of esophageal body waveform propagation upon provocation, including amplitude and duration, were significantly greater and prolonged in the neuropathology group (Table 2). Although, mid-esophageal provocations resulted in similar frequency recruitment of peristaltic reflexes (62.2% vs. 55.2%, neuropathology vs. control group, P=NS), the characteristic distribution of peristaltic reflex type, i.e., esophago-deglutition response (EDR) and secondary peristalsis (SP) were distinct. Comparisons between neuropathology (20.2% EDR, 79.8% SP) vs. controls (43.9% EDR, 56.1% SP) were different (P<0.0001).
Furthermore, the neuropathology group had lower frequency of deglutition apnea vs. control (19.3% vs. 30.0%, P=0.001) and lower frequency of respiratory rate changes (vs. control, 29.9% vs. 48.0%, P<0.0001).

**III. Effect of infusion media (air vs. liquids) and group (neuropathology vs. control) on the response latency and response duration of UES contractile reflex, Peristaltic reflex, and LES relaxation reflex**

We compared the response latency to the onset of the above adaptive reflexes between the neuropathology vs. control groups (Figure 4). Stimulations with the liquids (water and apple juice) did not yield any differences on any variables of interest; therefore, we combined them into liquid category, and data presented as such. Comparing air vs. liquid stimuli; liquid stimuli resulted in significantly longer response latencies to UES contractile reflexes as well as LES relaxation reflexes in both neuropathology and control groups. However, the response latencies to peristaltic reflexes were significantly longer with liquid stimulations vs. air in neuropathology group, but not so in the control subjects.

Between the groups comparisons, neuropathology group responded sooner (P<0.05) than controls, for UES contractile reflexes with air stimulation only, but not with liquid stimulation. On the other hand, response latencies to peristaltic reflexes were quicker in the neuropathology group with both air and liquid stimulations (vs. controls). No group differences for response latency were noted for LES relaxation reflex.
The durations of UES contractile reflexes (seconds) were similar between neuropathology vs. control groups respectively, with air stimulation (2.7 ± 0.4 vs. 2.7 ± 0.4, P=NS) and also with liquid stimulation (3.3 ± 0.2 vs. 2.9 ± 0.3, P=NS). In contrast, between the neuropathology vs. controls respectively, the magnitude of UES contractile reflexes (mmHg) was significantly higher with air stimulation (31.3 ± 2.0 vs. 20.8 ± 2.9, P=0.02) and also with liquid stimulation (31.8 ± 1.8 vs. 21.4 ± 2.4, P=0.003). On the other hand, the durations of LES relaxation reflexes (seconds) were similar between groups with air stimulation (neuropathology vs. control, 5.0 ± 0.5 vs. 4.6 ± 0.8, P=0.8) but significantly longer in the neuropathology subjects with liquid stimulations (5.6 ± 0.5 vs. 4.2 ± 0.6, P=0.03). The mean magnitudes of LES relaxation reflexes (mmHg) were similar between the neuropathology and control groups for both air (21.0 ± 1.2 vs. 22.0 ± 1.9, P=1.0) and liquid stimulations (23.4 ± 1.1 vs. 21.1 ± 1.4, P=0.6).

IV. Stimulus-Response Relationships between Neuropathology vs. Controls

The effect of physico-chemical characteristics of media (air vs. liquids-water and apple juice), graded volumes and group (neuropathology vs. control) on the frequency recruitment of adaptive reflexes of interest were investigated using multiple logistic regression model. The likelihood of enhanced recruitment of UES contractile reflexes, LES relaxation reflexes and peristaltic responses with increment in infusion volumes of air, water and apple juice was evident in both groups (Figure 5 and Table 3). Between the groups, differences for dose-response relationships were not evident.
DISCUSSION

Progressive brain maturation of feeding skills and aero-digestive reflexes is time-dependent and modified by co-morbidities (8, 11, 13, 14). Of relevance, neonatal neurological illness is associated with aero-digestive abnormalities including dysphagia, aspiration, gastroesophageal reflux, and life threatening airway events. The underlying mechanisms of malfunction of aero-digestive protective reflexes are unclear. In this study we investigated the effects of esophageal provocation on the physiology of UES, LES, and esophageal body reflexes. We summarize and explain the findings, discuss interpretation and implications as follows:

**Significant findings distinguishing the neuropathology group**

With middle esophageal provocation induced aero-digestive reflexes, the neuropathology group (contrasting controls) was distinct with the following salient differences: 1) UES resting tone, maximal tone and the magnitude of contractile response were all greater; 2) LES relaxation reflexes occurred earlier, and LES relaxation nadir durations were longer; 3) Higher frequency recruitment of peristaltic reflexes, in that secondary peristalsis and polymorphic waveforms dominated, and 4) there were less frequent deglutition response and less deglutition apnea. The physico-chemical characteristics of media (air or liquids) did not influence the frequency recruitment of these reflexes. 5) Esophageal body waveform amplitudes and peristaltic waveform durations were greater at all proximal-, middle- and distal- esophageal loci. These distinctive findings characterize the effects of global neonatal brain injury on aero-digestive reflex mechanisms.
The up-regulation of UES tone and reflex gain and increased inhibition of LES tone can be explained by a variety of mechanisms including changes in the central modulation of brainstem motor centers, changes in descending inputs (loss due to neuropathy, white matter injury, damage to basal ganglia, etc), or neuropathy of brainstem (reticular, premotor inputs to swallowing group motoneurons, etc). More detailed interrogation of neural pathways is needed to elucidate basic science mechanisms.

**Important similarities between the controls vs. neuropathology groups**

Important similarities were: 1) with UES contractile responses, both groups were able to distinguish response sensitivity between air vs. liquid stimuli, in that reaction with air was earlier than liquid. Stimulus dose-UES contractile reflex relationships were significant with air, water and apple juice. Response latencies and response durations were similar. 2) Response latencies to LES relaxation reflexes were prolonged with liquid than with air in both groups. The frequencies of occurrence of LES relaxation reflexes were volume-dependent and were significantly different for air, water and apple juice. Resting LES tones and magnitudes of LES relaxations were similar. 3) The frequencies of occurrence of peristaltic reflexes were volume-dependent and were significantly different for air, water and apple juice. 4) LES relaxation characteristics during esophago-deglutition response or secondary peristalsis were similar for most characteristics, and isolated differences may be related to volume effects.

The lack of differences is also an important finding, as neonatal period is a phase of rapid growth and development, and neurological recovery is possible. These
similarities in aero-digestive reflexes in the neuropathology group may constitute a potential mechanism for underlying neuroplasticity and recovery. Longitudinal studies are needed to ascertain sustained improvement and restoration of changes from the baseline.

**Interpretation of findings from neuropathology perspective**

The sensory and motor characteristics of UES contractile reflexes are distinct in the neuropathology group. Although threshold volumes with various stimulus modes were similar, quicker response latencies to UES contractile reflexes are suggestive of afferent neuronal hyper-excitability. Greater UES contractile properties are suggestive of increased efferent neuronal activation and therefore muscular contraction, such as may happen in spasticity. Indeed, the resting UES pressures were also greater in the neuropathology group. Furthermore, the sensory and motor characteristics of peristaltic reflexes were also distinct. A quicker response latency to air and liquid is suggestive of neuronal hyper-excitability and hyper-vigilant state. In addition, the discrepant proportion of greater secondary peristalsis suggests that peripheral reflexes are maintained and central reflexes (deglutition reflex) are impaired. Thus swallowing failure and airway provocation can happen in the presence of excessive secretions or feedings or more proximal GER events. Greater amplitudes and durations of pan-esophageal waveforms are suggestive neuromuscular hyper-excitability and hypertonic contractility. Under such conditions, esophageal peristaltic clearance can take longer. These effects may be modulated by both, peripheral and central vagal afferent-efferent pathways (4, 5, 19). LES relaxation reflex is facilitated by increased inhibitory neuronal activity to the
LES resulting in relaxation (6, 17). Changes with quicker and sustained fall in LES pressure from the resting tone are suggestive of increase inhibitory neuronal stimulation, and this mechanism is evident by the prolonged LES nadir and shorter relaxation duration. Collectively, these findings imply sustained and exaggerated increase in afferent as well as increased excitatory and inhibitory efferent outputs.

Middle esophageal stimulation induced response is mainly secondary peristalsis in adults, contrasting with occurrence of both esophago-deglutition response (defined as occurrence of deglutition with esophageal stimulation) and secondary peristalsis in healthy normal neonates (occurring in almost equal proportions). This pattern undergoes further normal maturation with growth and age, including LES relaxation characteristics during secondary peristalsis and esophago-deglutition response (7, 11, 24). In the current study, the frequency recruitment of secondary peristalsis was significantly (P < 0.0001) higher in neuropathology group (79.8%) compared with control group (56.1 %), implicating central brain stem effects, in that the discrepant proportion of greater secondary peristalsis suggests that peripheral reflexes are maintained and central reflexes (deglutition reflex) are impaired. Despite these differences, LES relaxation characteristics during esophago-deglutition response or secondary peristalsis were similar in many respects between controls and neurologically impaired subjects. These observations implicate inhibitory pathways governing LES relaxation were similarly stimulated whether by peripherally mediated (as in secondary peristalsis) or centrally mediated (as in esophago-deglutition response) mechanisms. These similarities aside, isolated differences in LES relaxation may be related to a) the effects of stimulus volumes or b) heterogeneity of neurological defects or c) heterogeneity
within the biological model studied or d) ongoing developmental influences regulating restoration of normality.

Pathophysiological Implications of this study

Paucity of central swallowing mechanisms such as primary peristaltic reflexes or esophago-deglutition reflexes is a marker of vulnerability of proximal aero-digestive tract (8, 11, 13, 14). Indeed, deglutition response is a chief response even in premature infants. Methods to improve sucking and swallowing skills need further investigation in neurologically impaired (1). Hypertonicity of UES and proximal esophageal body contractile waveforms suggest skeletal muscle dysfunction. In the presence of both, paucity of swallowing and exaggerated skeletal muscle tonicity, oral contents cannot undergo anterograde clearance, and poses a risky state for airway aspiration. Furthermore, the presence of increased inhibitory LES tone suggests that GER can occur (20-22); and at the same time, distal esophageal clearance can occur since LES can relax faster and for a longer period of time. Additionally, the presence of secondary peristalsis as a dominant esophageal clearance mechanism also supports that reflux clearance can still occur by this mechanism.

Heterogeneity within the neurologically impaired neonate likely explain some similarities (with control infant), and may be due to developmental influences regulating restoration of normality. These factors may be governed by neuroplasticity mechanisms that may aim to restore normalization. Because neonatal period is a dynamic growth phase and is confounded by maturation, longitudinal studies are needed to evaluate changes in sensorimotor characteristics and effects of neuroplasticity. The role of
sensory experiences and therapies in modulation and acceleration of safe feeding skills
during the period of dynamic somatic and brain growth in infants can be explored. Such
studies will help in further understanding of necessary neural pathways that may have
restored safe swallowing skills. Such studies will further clarify persistence or
amelioration of dysfunctional neuromotor markers, which may then serve as a prelude
to the development of translational clinical research protocols.

**Significance and Future directions**

Significantly, the provocative methodological approaches used to define the
neuromotor markers may be helpful in localizing deficits where dysphagia is a dominant
problem. Because neuropathic subjects commonly present with dysphagia and its
consequences, we specifically studied this group. The current study can also be the
basis for testing the integrity of peristalsis and gastro-esophageal junction function. We
speculate that such approaches when performed prior to and after the chronic medical
or surgical therapies may help clarify the therapeutic effects so as to help formulate
scientific basis for such interventions. As development of neurological disease is not a
static but dynamic problem, our approaches can also be useful to study maturational
changes in neuropathic subjects.

The next level of investigation in these neurologically impaired infants must
investigate longitudinal changes in specific neurological lesions so as to understand the
connectivity of networks pertinent to dysphagia and mechanisms of adaptation upon
esophageal provocation. This approach will then help identify the therapeutic targets at
the level of UES, LES or esophageal musculature in relation to reflexes, regulation of
tone, regulation of coordination, restoration of esophageal quiescence, as well as the mechanisms of symptom generation. Therapeutic strategies will then be possible, in addition to defining the physiologic basis for success or failure of therapies, which will in turn have significant implications. Larger studies are needed to discern the effects of potential confounders (gestational and postnatal maturity, pathologic basis of lesion, gender, associated deficits) on adaptive reflexes. Conversely, the effects of prevailing therapeutic strategies on aero-digestive adaptive reflexes in neuropathic infants can be investigated to clarify mechanisms of amelioration or lack thereof, given that the current study serves as a foundation for potential neuromotor markers.

In summary, this is the first study to interrogate the aero-digestive tract in neurologically vulnerable neonates. We clarified the sensorimotor pathophysiology of vago-vagal reflexes involved with esophageal clearance, airway protection and swallowing. Dysmotility mechanisms underlie in UES, esophageal body and LES contractile and relaxation properties. Although, perception to mid-esophageal provocation remains preserved in neurologically impaired neonates, sustained and exaggerated myogenic response from afferent activation is evident by: a) increased excitatory efferent outputs to the UES and esophageal body and b) increased inhibitory efferent outputs to the LES. Dysfunctional regulation of pharyngeal swallowing and infrequent deglutition responses indicate central malfunctions within the brain stem and vagal nuclei.

ACKNOWLEDGEMENTS

The authors are grateful for Michael Pizzuti, BS for technical support and Juan Peng,
MAS for statistical assistance.

GRANT

This work has been supported in part by the NIH grant RO1 DK 068158 to SR Jadcherla.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES


**FIGURE LEGENDS**

**Figure 1.** Esophageal manometry describing secondary peristalsis during mid-esophageal provocation. An example of UES contractile reflex, LES relaxation reflex and peristaltic reflex characteristics for neuropathy group (A) and control group (B). Higher resting UES pressure and longer LES nadir duration are noted in neuropathology group.

**Figure 2.** The resting UES pressure (A), maximum UES pressure (B), and UES contractile magnitude (C) upon esophageal provocation in the control vs. neuropathology group are shown.

**Figure 3.** The LES relaxation duration (A) and LES nadir duration (B) upon esophageal provocation in the control vs. neuropathology group are shown.

**Figure 4.** Response latency to UES contractile reflex (A), LES relaxation reflex (B), and peristaltic reflex (C) evoked upon esophageal provocation with regard to the type of media are shown. * P=0.02, † P=0.02, # P=0.02 for group difference.

**Figure 5.** Graded dose-response relationship of UES contractile reflex (A), LES relaxation reflex (B) and peristaltic reflex (C) in control and neuropathology groups is shown. P<0.05 for all media in both groups except for frequency of LES relaxation reflex with air in control group.
Table 1a. The characteristics of LES during esophageal provocation induced esophago-deglutition response.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Neuropathology</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of LES relaxation reflex, %</td>
<td>75.3</td>
<td>73.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Resting LES pressure, mmHg</td>
<td>19.3 ± 1.7</td>
<td>21.3 ± 1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>LES nadir pressure, mmHg</td>
<td>1.0 ± 1.0</td>
<td>1.8 ± 1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Response time to LES relaxation reflex, s</td>
<td>3.9 ± 0.3</td>
<td>4.0 ± 0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>LES relaxation time, s</td>
<td>3.0 ± 0.5</td>
<td>4.1 ± 0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>LES nadir duration, s</td>
<td>6.9 ± 0.9</td>
<td>4.8 ± 0.9</td>
<td>0.09</td>
</tr>
<tr>
<td>LES relaxation pressure drop, mmHg</td>
<td>22.2 ± 1.5</td>
<td>23.2 ± 1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Control</td>
<td>Neuropathology</td>
<td>P-value</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Frequency of LES relaxation reflex, %</td>
<td>57.6</td>
<td>55.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Resting LES pressure, mmHg</td>
<td>17.7 ± 1.1</td>
<td>17.3 ± 1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>LES nadir pressure, mmHg</td>
<td>2.0 ± 0.6</td>
<td>3.6 ± 1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Response time to LES relaxation reflex, s</td>
<td>3.7 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>LES relaxation time, s</td>
<td>2.2 ± 0.2</td>
<td>3.2 ± 0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>LES nadir duration, s</td>
<td>4.8 ± 0.3</td>
<td>3.6 ± 0.5</td>
<td>0.04</td>
</tr>
<tr>
<td>LES relaxation pressure drop, mmHg</td>
<td>22.6 ± 1.0</td>
<td>20.3 ± 1.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 2. The characteristics of esophageal waveform propagation upon provocation between neuropathy and control subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Neuropathy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude of proximal esophagus, mmHg</td>
<td>36.3 ± 2.8</td>
<td>45.3 ± 2.0</td>
<td>0.008</td>
</tr>
<tr>
<td>Duration of proximal esophagus</td>
<td>3.8 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>propagation, s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude of middle esophagus, mmHg</td>
<td>52.2 ± 3.2</td>
<td>72.8 ± 2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of middle esophagus</td>
<td>3.8 ± 0.4</td>
<td>5.7 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>propagation, s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude of distal esophagus, mmHg</td>
<td>44.9 ± 3.9</td>
<td>60.3 ± 2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of distal esophagus</td>
<td>4.3 ± 0.5</td>
<td>5.5 ± 0.3</td>
<td>0.07</td>
</tr>
<tr>
<td>propagation, s</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Graded dose-response relationship across media and groups.

<table>
<thead>
<tr>
<th>Frequency, %</th>
<th>Media</th>
<th>Control Odds Ratio (95% CI)</th>
<th>P-value</th>
<th>Neuropathology Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UES contractile reflex</td>
<td>Air</td>
<td>1.7 (1.3-2.4)</td>
<td>0.0008</td>
<td>1.5 (1.1-2.0)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>2.6 (1.6-4.4)</td>
<td>0.0002</td>
<td>3.4 (2.3-4.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Apple Juice</td>
<td>2.9 (1.6-5.0)</td>
<td>0.0002</td>
<td>3.8 (2.0-6.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Liquids</td>
<td>2.7 (1.9-3.9)</td>
<td>&lt;0.0001</td>
<td>2.7 (1.9-3.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LES relaxation reflex</td>
<td>Air</td>
<td>1.3 (1.0-1.5)</td>
<td>0.02</td>
<td>1.3 (1.1-1.7)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3.2 (1.7-6.2)</td>
<td>0.0005</td>
<td>3.9 (2.5-6.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Apple Juice</td>
<td>2.9 (1.4-6.2)</td>
<td>0.006</td>
<td>4.3 (2.6-7.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Liquids</td>
<td>3.0 (1.7-5.0)</td>
<td>&lt;0.0001</td>
<td>4.1 (2.7-6.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peristaltic reflex</td>
<td>Air</td>
<td>1.3 (1.1-1.5)</td>
<td>0.0007</td>
<td>2.9 (0.5-17.1)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>23.2 (5.9-91.6)</td>
<td>&lt;0.0001</td>
<td>25.8 (7.2-91.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Apple Juice</td>
<td>5.2 (1.4-19.8)</td>
<td>0.01</td>
<td>45.1 (7.5-272.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Liquids</td>
<td>1.8 (1.3-2.6)</td>
<td>0.002</td>
<td>4.6 (2.4-8.7)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Logistic regression and GEE methods were applied to evaluate the relationship between the response (binary outcome of the three specific reflexes, i.e., UES contractile reflex, LES relaxation reflex, peristaltic reflex) and the independent variables (media, and graded volume). Significant odds ratio indicate a positive correlation between volume increments and the predictive reflex. For example, with a unit increase in dose volume of air, the occurrence of UES contractile reflex was 1.7 in control group.
A. Response latency to UES contractile reflex

Neuropathology

Control

P<0.0001

P=0.03

Time (seconds)

B. Response latency to LES relaxation reflex

Neuropathology

Control

P<0.0001

P=0.004

Time (seconds)

C. Response latency to peristaltic reflex

Neuropathology

Control

P=0.02

Time (seconds)

Liquids

Air