A Safe and Noninvasive Test for Vagal Integrity Revisited

Nagammapudur S. Balaji, MS, FRCS; Peter F. Crookes, MD; Farzaneh Banki, MD; Jeffrey A. Hagen, MD; Joy E. Ardill, PhD; Tom R. DeMeester, MD

Hypothesis: Measurement of pancreatic polypeptide (PP) response to sham feeding and pharmacological stimulation is a safe, noninvasive, and sensitive test for vagal integrity.

Design: Interventional study with control arms.

Setting: Tertiary center for esophageal surgery.

Patients: Thirty healthy volunteers and 25 patients who underwent total esophagectomy formed the control group with intact vagi and known vagotomy, respectively.

Intervention: Blood samples were obtained 15 minutes before and immediately before sham feeding to determine basal PP levels. Samples were also obtained 15, 30, 45, and 60 minutes after the sham feeding and 10 and 20 minutes after administration of 5 mg of intravenous edrophonium hydrochloride.

Main Outcome Measure: Pancreatic polypeptide response to sham feeding and edrophonium administration were compared in both groups and the optimal percentage of rise from basal levels with maximal sensitivity and specificity was determined.

Results: Basal levels were similar in both groups (50 vs 45 ng/L). The maximum percentage of rise within 30 minutes after sham feeding was significantly higher in healthy subjects than in patients who underwent vagotomy ($P<.001$). A rise of 50% was seen in 24 (83%) of the 29 healthy subjects vs 2 (8%) of the 25 patients who underwent vagotomy ($P<.001$). This rise in PP level had a sensitivity of 83%, specificity of 92%, and a positive predictive value of 92% for identifying an intact vagus. The administration of edrophonium did not improve these results.

Conclusions: A rise of more than 50% in the PP level within 30 minutes of sham feeding is a strong indicator of vagal integrity. This test has the potential to investigate vagal injury after gastroesophageal surgery.

Arch Surg. 2002;137:954-959

Increasing acceptance of the value of antireflux surgery in gastroesophageal reflux disease has resulted in a concomitant increase of postoperative symptoms such as bloating, abdominal pain, flatulence, and diarrhea that are often assumed to be adverse effects of surgery. It is unclear if such symptoms are the result of an underlying diffuse gastrointestinal (GI) motility disorder that has been unmasked by the surgery, physiologic consequences of the altered geometry of the cardia, or an error in the technical performance of the procedure. One potential cause of the symptoms is damage to the vagus nerves as they pass with the esophagus through the esophageal hiatus. This would result in loss of motor innervation to the antrum and pylorus, and loss of receptive relaxation in the body and fundus. This is difficult to measure clinically. Testing of vagal function has fallen out of the repertoire of most GI surgeons because elective vagotomy is so rarely performed. Consequently, there is no agreed on method of determining if the vagal nerves have been damaged, when faced with a patient reporting the aforementioned symptoms after antireflux surgery.

See Invited Critique at end of article

Historically, the acid secreting capacity of the stomach has been used as a surrogate for vagal function, by quantifying acid secretion in response to vagal stimulation, or by spraying Congo red onto the gastric mucosa. These methods are invasive and require either endoscopy or intubation of the stomach. In addition, the response to Congo red is subjective and qualitative. There is, therefore, a need for...
PATIENTS AND METHODS

The group with intact vagi included 30 healthy volunteers (male-female ratio, 16:14) with no history of upper GI disease or prior GI surgery. The median age (interquartile range [IQR]) was 31 (26–36) years. They were enrolled in this study after the completion of a standard health questionnaire.

The group with established vagotomy was composed of 25 patients (male-female ratio, 18:7) who had esophagectomy for benign or malignant disease of the esophagus. All had deliberate transection of the vagus nerve trunks. The median age was 63 (53–70) years. Patients who had diabetes mellitus, bronchial asthma, abnormalities of cardiac rhythm, and mechanical intestinal or urinary tract obstruction were excluded from the study. A colon interposition was performed in 13 patients, while 12 patients had gastric interposition for reconstruction of the esophagus. The institutional review board at the University of Southern California, Los Angeles, approved the study prior to commencement.

STUDY PROTOCOL

Subjects were required to fast for at least 6 hours before the start of the study. After obtaining intravenous access, 2 samples of 5 mL of venous blood were obtained 15 minutes apart before the commencement of a sham feeding. The subjects then underwent a standardized sham feeding using a “chew and spit” technique during a 15-minute period. The food consisted of a standard hamburger, fries, and a drink obtained from the same fast-food establishment. They were specifically instructed to avoid any food or drink going into the stomach. Four samples of 5 mL of venous blood were then serially obtained at 15, 30, 45, and 60 minutes after the sham feeding.

Edrophonium hydrochloride, 2.5 mg, was then administered intravenously with the subject connected to a pulse oximeter and an electrocardiographic monitor. A further 2.5 mg of edrophonium hydrochloride was administered if no adverse effects were observed in 30 seconds. Two further samples of blood were obtained at 10 and 20 minutes after the administration of edrophonium. Atropine was kept ready for administration to counteract any abnormalities of cardiac rhythm. The blood samples were immediately centrifuged after the completion of the study to separate plasma (average, 2 mL) that was stored at −72°C in separately labeled containers and transported in dry ice to the Peptide Laboratory, Institute of Clinical Medicine, Queens University of Belfast, Belfast, Northern Ireland.

ANALYSIS OF SAMPLES

BY RADIOIMMUNOASSAY TECHNIQUE

The PP polyclonal antibody was raised in rabbit to human PP. It reacted with the N-terminal region of human PP. Pancreatic polypeptide was labeled with sodium iodine I 125 (Amersham Biosciences, London, England) using the iodogen method. The radiolabeled tracer was purified by reverse phase high-performance liquid chromatography. A Bondapak C18 column was used and the separation based on the principle that some peptides are more hydrophobic than others. A radiolabeled material of high specific activity was obtained that constituted the tracer. Human PP was used as the standard and a calibration curve constructed between the concentrations 625 ng/L and 19 ng/L. Pancreatic polypeptide was measured in plasma, which had been extracted using alcohol. To 1 mL of plasma, 1.6 mL of ethanol was added; this precipitated larger proteins that interfere with the assay. The precipitate was then removed and the alcohol evaporated through a current of air.

The extract was reconstituted in buffer at pH 7.4 for assay. Assay conditions were 2-day preincubation (antibody and sample) and 2-day postincubation tests with radiolabeled tracer (all at 4°C). Separation of free fraction from bound fraction was achieved using dextran-coated charcoal. The tubes were counted on a gamma counter and the values calculated. Interassay variation was 8.8% at 150 ng/L and intra-assay variation was 5.6% at 150 ng/L. Results are expressed in nanograms per liter (1 ng/L = 0.24 pmol).

STATISTICAL ANALYSIS

Data analysis was done using Prism software Version 3.02 (Graph Pad Inc, San Diego, Calif). The Wilcoxon matched-pairs test was used to compare samples in individual patients. Comparison between the groups was done using the Mann-Whitney test. Proportions were compared using the Fisher exact test. \( P < .05 \) was statistically significant.

a noninvasive and quantitative test of vagal function to elucidate vagal integrity when faced with a patient reporting upper GI symptoms after antireflux surgery. Pancreatic polypeptide (PP) response to vagal stimulation using sham feeding and insulin-induced hypoglycemia has been used to check completeness of vagotomy. \(^{8-10} \) but did not gain widespread acceptance. The exact mechanism and pathway of PP secretion was unclear and the emergence of highly selective vagotomy, and the later the disappearance of vagotomy altogether, rendered it largely irrelevant. \(^{3,11} \)

Pancreatic polypeptide is a 36 amino acid polypeptide hormone first discovered as a contaminant in insulin preparations. \(^{12} \) The pathways governing the secretion of PP have been worked out. \(^{13,14} \) The secretion of PP is affected by several physiologic stimuli, but the principal regulatory mechanism is vagal cholinergic stimulation of the gastric antrum, causing release of a blood-borne PP-releasing factor that mediates its secretion from the pancreas. It is of importance that the same branches of the vagal nerve that mediate PP release also control antral and pyloric motility. It does not require the presence of pancreatic vagal innervation, since total extrinsic pancreatic denervation did not significantly alter the PP response to a meal in dogs. \(^{15-17} \) Pancreatic polypeptide secretion after a meal is known to be characteristically biphasic. \(^{13,14} \) An early primary response that is vagally mediated occurs 10 to 30 minutes after stimulation, and a more prolonged secondary phase occurs after 30 minutes to 6 hours. The secondary phase is not totally dependent on vagal activity but is also produced by enteropancreatic and neurohumoral pathways.

Both sham feeding and insulin-induced hypoglycemia have been used to produce vagal stimulation. Insulin-induced hypoglycemia has been abandoned in the United States be-
cause of its risk of serious cardiac or cerebral insults, although it remains in use in Europe.18,19 Response to sham feeding has been problematic because of the wide range reported for healthy subjects. It is also a less powerful stimulus of PP release than hypoglycemia. Consequently, in the case of diminished or absent response to vagal stimulation, it is difficult to distinguish vagal damage from intrinsic pancreatic lack of PP. We hypothesized that sham feeding—induced PP response might be more meaningful if the capacity of the pancreas to secrete PP were known. We postulated that short-acting cholinergic stimulation, using the agent typically used for the diagnosis of myasthenia gravis (edrophonium hydrochloride), would give a measure of the maximum secretory capacity of the pancreas. This would be analogous to performing pentagastrin stimulation after measuring the acid output in response to sham feeding. Hence, we sought to assess the response of PP to sham feeding in healthy subjects and patients with known vagal transection to define a response that would indicate with confidence that the vagi were intact.

The study was successfully completed in all the subjects.

**RESULTS**

**BASAL LEVELS OF PP**

Basal levels were calculated as the average of the 2 levels taken before the sham feeding. They were similar in the healthy subjects (median, 50 ng/L; IQR, 40-112 ng/L) and the vagotomy group (median, 45 ng/L; IQR, 33-62 ng/L) (P=.13).

**LEVELS OF PP AFTER SHAM FEEDING**

A rise in PP levels from basal was seen in 27 (93%) of the 29 healthy subjects and in 11 (44%) of the 25 subjects after vagotomy following sham feeding. The higher of the 2 levels in the first 30 minutes after the sham feeding was considered as the “cephalic peak response” and used for data analysis. The median cephalic peak response levels were significantly higher in the healthy subjects (median, 130 ng/L; IQR, 80-227 ng/L) when compared with the vagotomy group (median, 55 ng/L; IQR, 40-77 ng/L) (P<.001). The response in the vagotomy group 30 minutes after the sham feeding was also significantly different between the 2 groups (P<.005). This response observed in the second 30 minutes might be attributed to the enteric phase that was not entirely dependent on the vagal stimulation; hence, this part of the study was not considered for data analysis.

A scatter plot of individual cephalic peak responses in both the groups is shown in Figure 1. A considerable overlap is observed between both groups when either the 75th or 95th percentiles of the basal levels of healthy subjects were considered for differentiation of vagal nerve status with regard to PP response. The cephalic peak response phase was also assessed by calculating the percentage of rise from basal values, enabling each subject to serve as his or her own control. A median percentage of rise of 100% (IQR, 58-210 ng/L) was observed in the healthy subjects, in marked contrast with 0% (IQR, -31 to +35 ng/L) in the vagotomy group.

**PLOT OF RECEIVER OPERATING CHARACTERISTIC CURVE**

A receiver operating characteristic curve was constructed for various thresholds of the percentage of rise in PP level (10%-250%) (Figure 2). The optimal threshold value with the combined maximal specificity and sensitivity was found to be a 50% rise from basal values (sensitivity, 83%; specificity, 92%; and positive predictive value, 92%).

Figure 3 shows the scatter plot for the percentage of rise from basal values for controls and vagotomized subjects in relation of the 50% threshold obtained form the receiver operating characteristic curves. The enhanced discriminating capacity of the test is well seen.

**RESPONSE AFTER EDROPHONIUM ADMINISTRATION**

The median maximal PP response after edrophonium stimulation in the healthy subjects was 155 ng/L (IQR, 105-232 ng/L). This was higher than the maximal response in subjects after vagotomy 90(IQR, 37-130 ng/L) (P<.003). However, only 17 (57%) of the healthy subjects and 12 (47%)...
of the patients who underwent vagotomy exhibited any rise in PP level after the administration of edrophonium with respect to basal values. Furthermore, PP levels after sham feeding were higher than the edrophonium-stimulated values in 52% of the healthy subjects and 49% of subjects after vagotomy. As a result, edrophonium stimulation did not improve the diagnostic value of the test.

**COMMENT**

The incidence of symptoms such as early satiety, postprandial fullness, epigastric discomfort, and diarrhea have been reported in up to 30% of subjects after antireflux surgery. The pathogenesis of these symptoms remains a matter of dispute, but one potential cause is inadvertent vagal damage at the time of surgery. The actual incidence of vagal damage after antireflux surgery is completely unknown. The availability of a test that can document vagal integrity would be helpful in many ways. It may help resolve legal disputes when a disgruntled patient alleges technical error in performance of the operation. It would also greatly enhance the surgeon’s ability to evaluate the status of the vagus nerve before a second antireflux surgery during which there is a higher risk for vagal damage.

The concept of PP response to sham feeding is not new. However, we attempted to overcome the deficiencies of previous studies by expressing the PP response as a percentage over basal level, thus removing the variability caused by the wide range of normal values. We also attempted to enhance the accuracy of the test by relating the response to edrophonium stimulation. We used a receiver operating characteristic curve to define the optimal cutoff point, finding that a 50% rise from basal values best discriminates patients with vagotomy from healthy subjects. However, it is true that 17% of the healthy subjects did not exhibit this degree of response. We had hoped that these subjects would be identified by a lack of response to edrophonium administration, but the addition of edrophonium did not enhance the discriminatory ability of the test since 2 of the 5 healthy subjects with no response to sham feeding had a notable response to edrophonium administration. The best way to identify nonresponders would be to study every patient preoperatively. At this point we are unable to explain why 2 (8%) of patients after esophagectomy had a significant PP level rise after sham feeding. Possible explanations are that (1) 1 or more vagal divisions were not transected, perhaps because they branched more proximally or (2) the patients actually swallowed some of the meal and stimulated the antrum directly by an extravagal mechanism.

**CONCLUSIONS**

Pancreatic polypeptide response to sham feeding is a dependable test of vagal integrity. The optimal threshold seems to be more than 50% rise from basal levels. This test can be applied to patients before and after repeated antireflux surgery, primary antireflux surgery, and vagal-sparing esophagectomy when the integrity of the vagus nerve is in question.

*This paper was presented at the 73rd Pacific Coast Surgical Association Meeting, Las Vegas, Nev, February 16, 2002,* and is published after peer review and revision. The discussion is based on the originally submitted manuscript and not the revised manuscript.

Corresponding author: Peter F. Crookes, MD, Department of Surgery, University of Southern California, Los Angeles, CA 90033 (e-mail: pcrookes@surgery.usc.edu).

**REFERENCES**

on a reexamination of the measurement of plasma PP levels in
intent of the assessment of vagal function.

cation of fundoplication. This is a fundamental difference in the
cination of inadvertent vagal nerve transection or injury as a compli-
cation of completeness of vagotomy, but rather in the detec-
tion of laparoscopic fundoplication for gastroesophageal re-
diagnosing incomplete vagotomy following failed operation for
application of pH indicator dyes such as Congo red have all been
means of insulin-induced hypoglycemia, acid secretagogues (eg,

Bruce E. Stabile, MD, Torrance, Calif: I really would like to
ho Dr Wilson’s comment that this was a beautifully pre-
ented and wonderfully illustrated presentation. I would like
to congratulate Drs Balaji and coworkers on this work.

The clinical assessment of vagal nerve integrity following sur-
ery for acid-peptic disease and other gastroesophageal maladies
has always been and remains a highly vexing problem. Testing by
means of insulin-induced hypoglycemia, acid secretagogues (e.g.,
histamine, betazole, and pentagastrin), and endoscopic topical
application of pH indicator dyes such as Congo red have all been
used but are either dangerous or uncomfortable for the patient.
As most of these tests were developed with the specific intent of
diagnosing incomplete vagotomy following failed operation for
peptic ulcer disease, their use in recent years has all but disap-
ppeared. However, with the recent dramatic increase in the appli-
cation of laparoscopic fundoplication for gastroesophageal re-
flux disease, there is a renewed interest in the assessment of vagal
integrity. As opposed to years past, interest is not in the deter-
mination of completeness of vagotomy, but rather in the detec-
tion of inadvertent vagal nerve transaction or injury as a compli-
cation of fundoplication. This is a fundamental difference in the
intent of the assessment of vagal function.

It is within this context that the authors have embarked on a reexamination of the measurement of plasma PP levels in
response to sham feeding. While sham feeding–stimulated PP
release is not a new test for the assessment of vagal integrity, the
authors have advanced its utility and efficacy by carefully
defining a threshold level to separate individuals with normal
intact vagal nerves from those with complete or total va-
gotomy following esophagectomy.

The findings of the study as presented are clear; however, the authors’ interpretation of the results and their conclusions
may be overly optimistic. With appropriate methods their data
analysis indicated that a 50% rise in plasma PP level above basal
within 30 minutes after sham feeding was present in 24 (83%)
of 29 of the healthy subjects but only in 2 (8%) of 25 of those
with total vagotomy. The 18% of the false-negative rate for in-
 tact vagal innervation means that 1 in 6 patients could be wrongly
interpreted as having nonintact vagal innervation if tested only
postoperatively.

The major shortcoming of the study presented is that the au-
 thors have not investigated in any way the efficacy of the test in
patients with only partial vagal integrity, that is, in those with un-
intended partial vagotomy. Remember, this is precisely the group
that the authors seek to identify using the test. Patients who have
experienced a technical misadventure involving the vagal nerves
during fundoplication are likely to have had a partial or incom-
plete rather than a total or complete vagotomy. As no such patients
were included in the study, the authors can only reasonably con-
clude that the test discriminates between those with normal va-
gal innervation and those with total absence of vagal innervation.
More specifically, the authors have demonstrated only that the
PP response to sham feeding accurately identifies approximately
5 of 6 individuals with all vagal innervation intact and approxi-
mately 11 of 12 individuals with no remaining vagal innervation.
They have presented no data on partial or, if you will, single nerve
vagotomy. Therefore, the applicability of the test in the postop-
erative setting using the authors’ threshold criterion remains un-
tested as few, if any, patients will have had unintentional total or
complete vagotomy as a result of fundoplication. Thus far, we have
been shown only the results from the all or none extremes of va-
gal innervation. For the test to be clinically useful, it must be able
to discriminate between patients with entirely intact and only par-
tially impaired vagal function. I would like to ask the authors if
they believe the test is specific enough to identify patients with
only partial vagotomy, and whether they now have any informa-
tion to substantiate this belief.

My final question further relates to this conundrum regard-
ing the patient with only a partial vagotomy. How do the
authors intend to establish the validity and efficacy of their test-
ing method in patients with a vagal injury that is less than total
vagotomy? I would suggest that they approach this in 3 ways.
First, study the problem in an appropriate animal model such as
the dog. Second, test any of the now rare patients who pre-
 sent with recurrent ulcer following truncal vagotomy, and most
particularly, any such patient who is to undergo reoperation
where there will be opportunity to confirm an incomplete va-
gotomy. Third, a series of previously unoperated-on patients
who are about to undergo fundoplication should be tested pre-
operatively and then postoperatively in a prospective trial. Cor-
relation of postoperative symptoms and signs suggesting va-
gal injury can then be correlated with the preoperative and
postoperative test results.

In conclusion, I would like to congratulate the authors on a
well-conducted study designed to address a timely clinical
issue. It is my hope that future work will be undertaken to de-
terminate whether this test can reliably discriminate between pa-
teins with normal vagal integrity and those with partial vagal
denervation due to operative injury.

Dr Crookes: I would like to thank Dr Stabile for his in-
sightful comments and, indeed, many of them are actually ad-
dressed in the “Comment” section of our paper. He is com-
pletely correct in that yes, we only did test those whom we
thought had the most complete vagotomy we could imagine,
namely, an esophagectomy. Those are the patients that we have
access to in our practice. I cannot remember the last time I saw
a patient with a recurrent duodenal ulcer in whom the possi-
bility of incomplete vagotomy arose.

So I think the core of the discussion today is the fact that
we have presented no data that will tell you whether the vagus
has been partially damaged or not. But I would remind you that
partial damage to the vagus nerve or damage for example to
only 1 trunk may be completely irrelevant to the postopera-
tive symptoms. The patients whom we are talking about who
are coming back to their physicians reporting these abdomi-
nal symptoms and their consulting lawyers reporting that the
surgeon damaged their vagus nerves probably have had a com-
plete vagotomy. Many years ago in the development of highly
selective vagotomy, and especially in the early laparoscopic era,
it was common practice deliberately to transect the posterior
vagus as part of a highly selective vagotomy. Those patients did
not have a pyloroplasty and probably you need to have both vagi
damaged before you have the symptoms resulting from it.

While I agree that it is a weakness of our study that we can-
not distinguish complete from partial vagotomy, it also may be
the fact that we do not really need to distinguish it since only
a complete vagotomy may be responsible for their symptoms.
My suspicion is, and of course future work will confirm or re-
fute this, that minor degrees of vagal injury such as may have occurred in the course of fundoplication will not be responsible for the sustained symptoms that the patients report.

One of the things that stimulated this work for us was the fact that I had heard of several patients who were suing their surgeons because this notion has been raised, and there was no agreed method of trying to find a parameter that could clear the surgeon, something that would establish whether the vagal nerves were complete or not. Several significant laboratories on the West Coast were unable to offer such a test and this was a stimulus for me to think up a method of trying to identify quantitatively and objectively if the vagal nerves were preserved.

Dr Stabile is also quite correct in that the ideal way to do this would be to test the patients preoperatively. Since it is very simple and noninvasive and the only difficulty is avoiding swallowing a very tasty meal and having to spit it out. The only difficulty is coming and having a couple of blood samples obtained. If this test turns out to be worthwhile, it may be that we may be able to simplify the blood testing required so that only 1 or 2 blood samples would be sufficient instead of the 8 that we calculated for our protocol.

So I would like to thank you all for your comments and to reiterate the point that it is preliminary work and that the subjects that Dr Stabile has indicated for our future work may be the subject of future presentations at this meeting in future years.

Invited Critique

Gastrointestinal physiology has had several topics that have perennial interest, including this update on a method for defining the integrity and functional status of the vagus nerve(s). The current report on PP is welcome for several reasons, including the opportunity to consider aspects of vagus nerve function as a topic for the clinical laboratory. The functional integrity of vagus nerves, the immediate focus of investigation, is a question that may arise in postoperative patients who are experiencing digestive tract difficulty; the putative explanation that “damaged vagus nerves” are responsible should be carefully considered, however, as this study indicates.

The wide variations in serum PP levels in the basal and stimulated state (Figures 1 and 3 of the Balaji et al article) are at the heart of the fact that PP levels cannot be a reliable indicator of the status of an individual patient’s vagus nerve status. The sophisticated analysis of population or interquartile means and the receiver operating characteristic curve may well be useful for showing the population dynamic, but are not useful for interpreting the status in any one person. Perhaps an unanticipated benefit of these data is to underscore the fact that each individual has a unique PP response, and that any conclusions must be made in a tentative fashion; this fact should also be kept firmly in mind by those who counsel counselors regarding the implications of PP levels.

Finally, the failure of the administration of edrophonium to provide a predictable result is not surprising, given the fact that mild cholinergic stimulation has never been found equivalent to maximal vagus–mediated stimulation, which is the end-result of a whole family of peptide and hormonal neurotransmitters. The vagus nerve does not easily reveal its status at times, unfortunately for those of us who attempt to provide reasonable explanations for our patients.

Philip E. Donahue, MD
Chicago, Ill