Tracking the Moveable Feast: Sonomicrometry and Gastrointestinal Motility

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Ultrasonomicrometry measures distance between piezoelectric crystals based on transmission time of ultrasound bursts. It allows monitoring of coordinated motion of small and delicate tissues, including gastrointestinal sphincters. Its suitability for motility studies in small animals such as mice suggests that its use in gastrointestinal studies will increase in coming years.

Ultrasonomicrometry has long been used in cardiovascular and other branches of physiological research but has been used only rarely to study gastrointestinal (GI) motility. Recent technological improvements have rendered it more suitable for GI studies. This review will focus on the properties of ultrasonomicrometry relevant to its use in the study of GI motility.

The mammalian GI tract is invested with an intrinsic enteric nervous system consisting of an enormous number of neurons (~10⁸ in humans), comprising 14 presently identified functional classes (10). The predominant role of that portion of the enteric nervous system located in the myenteric plexus, situated between the longitudinal and circular smooth muscle layers of the digestive tube, is to coordinate enteric smooth muscle activity to effect gut motion. The presence of such a massive complement of intrinsic neuronal "hardware," along with the vast array of endocrine, paracrine, and extrinsic neural signals that regulate it, indicates an immense motor repertoire and extensive coordination of movement throughout the GI tract. It also suggests a high degree of plasticity and learning in the gut. Ironically, our ability to study and describe the complexity of the systems controlling gut motion is developed far more than our ability to study that motion itself.

A large body of GI research is concerned with the study of motility, defined by Stedman's Medical Dictionary as, "the power of spontaneous movement." However, with the exception of imaging studies (ultrasonography, MRI, computed tomography, X-ray), most research techniques used to detect and quantify motility do not measure movement directly but instead measure concomitants of gut motion. Such methods include those that detect smooth muscle electrical activity (electromyogram; EMG), tension/force developed by smooth muscle contraction (force transducers), changes in intraluminal pressure resulting from GI motion (including multilumen catheter and Dentsleeve methods), and transit of intraluminal contents along the length of the GI tract and/or across distinct boundaries (e.g., the pylorus, the ileocolic sphincter, or the anus). Due to the complementary strengths and weaknesses of various methods for detecting gut motion and its impacts, combinations of methods are frequently used (8, 13).

Ultrasonomicrometry provides a means of directly measuring the motion of soft tissues at a number of discrete locations, continuously and with high spatial and temporal resolution. It is particularly well suited to detecting motion of small structures such as sphincters, registers both tonic and phasic changes, can detect both contraction and expansion or stretching, can be used in either fed or fasted animals, and is suitable for use in small experimental animals, including mice.

Basic principles

The physical basis of ultrasonomicrometry is the measurement of the time required for an ultrasound signal to travel between a transmitter and a receiver each affixed to the tissue whose motion is to be studied. If the speed of propagation of the sound wave is known and is constant in the medium through which it travels, then the distance between the transmitter and the receiver can be calculated from this time measurement. On the basis of measurements from a number of mammalian soft tissues at physiological temperatures, a transit speed of 1.59 mm/ μ s is typically used for this calculation. The transmitted signal is produced by energizing a piezoelectric crystal, which leads to physical distortion of the crystal, thereby producing an ultrasonic sound wave. This wave propagates through the aqueous (or soft tissue) medium and impinges on a second, receiving piezoelectric crystal, which is distorted by the sound and thus produces a corresponding voltage change. The time of arrival of the wave at the receiving crystal is taken as the time at which the voltage change produced by the receiver exceeds a user-adjustable threshold. The transmitting crystal is energized at a known time according to a fixed cycling rate, the time of arrival of the impulse at the receiving crystal is registered, and the distance between transmitter and receiver during that interval is calculated from the time difference. Typical sound frequencies used in modern devices are on the order of 1–3 MHz. Typical cycling rates used in cardiovascular, respiratory, and skeletal muscle studies range between 50 and 2,000 Hz/crystal. Distance measurements are made continuously between pairs of crystals affixed to the tissue(s) of interest. The dynamic resolution of these distance measurements can be as small as 0.016 mm. Crystals may be affixed by using cyanoacrylate tissue glue or with sutures manufactured into the epoxy bead surrounding

the crystal. The smallest crystal head sizes presently available are 0.7 mm in diameter.

In any given preparation many crystals may be used, thus allowing for many essentially simultaneous measurements between crystal pairs. Digital sampling techniques have increased the maximum number of crystals that can be used simultaneously. In appropriately outfitted systems, up to 32 crystals may be used in a single preparation, with distances between all pairs of crystals measured. Software is available that allows three-dimensional reconstruction of the positions of all crystals in the array, thus permitting determination of the enclosed volume.

Ultrasonomicrometry should not be confused with ultrasonography. The former uses transmitters and receivers directly affixed to the structures whose motion is being analyzed. The latter is an imaging technique in which structures are scanned by using an ultrasound probe and imaged as a result of differences between the acoustic properties of the tissues, including the manner in which they reflect, or echo, ultrasound. As with any imaging technique, skill and experience in interpretation of the visual information is necessary, and analysis of the motion of particular points relative to each requires the presence of distinctive landmarks. Ultrasonography has been used to study gastric circumference, volume, and transpyloric flow in conscious human subjects (4).

Recent studies

We have recently taken advantage of sonomicrometry to examine the coordinated motion of the stomach of urethaneanesthetized rats in response to intravenous bolus injection of cholecystokinin (CCK), a peptide hormone that is normally released in response to the presence of particular nutrients, including lipids, in the proximal duodenum and is responsible for delaying further gastric emptying (1). The impact of intravenous CCK on gastric motility has been previously investigated by using a variety of methods, including EMG, manometry, strain gauge recordings, and imaging studies, and thus the model provided an excellent opportunity to compare results with those obtained by using complementary methods. Pairs of crystals were oriented parallel to the circular muscle and affixed to the serosa of the lower esophageal sphincter (LES), the corpus, the antrum, and the pylorus (Fig. 1). In some experiments, a triad of crystals in corpus were used to measure both circular and longitudinal motions. Gastric intraluminal pressure was measured simultaneously by using a pressure transducer placed via an incision in the nonglandular portion of the stomach into the distal corpus, the duodenum was ligated, and a basal pressure of 4-6 cmH₂O was established via intragastric injection of saline. Under these conditions, intravenous injection of CCK octapeptide (CCK-8) caused a doserelated (0.3-3 µg/kg) drop in intragastric pressure and an inhibition of rhythmic (5.7 \pm 0.3/min, n = 9) gastric contractile activity, beginning simultaneously with contraction of pylorus and antrum and relaxation/opening of LES (Fig. 2). Motions of corpus were small and not significantly different from no response (vehicle controls). Motion of circular corpus was smallest of that in any region, and the direction of the



FIGURE 1. Schematic representation of the ultrasonometric piezoelectric crystal positions on the stomach and sphincters. Dotted lines indicate crystal pairs from which distance data were recorded and are not physical structures. LES, lower esophageal sphincter.

response (contraction or expansion) varied between preparations, although it was constant and graded with dose within a given preparation. The overall pattern of response observed was consistent with retropulsion of gastric contents, with pressure drops resulting at least in part due to the relaxation and opening of LES. The CCK_A receptor antagonist devazepide significantly reduced the duration, maximum amplitude, and integrated response to intravenous CCK-8 for all motion traces and reduced the duration and integrated response seen in the intragastric pressure trace. However, the maximum amplitude of intragastric pressure drop was not significantly reduced. This was interpreted as a result of a combination of the common cavity effect during the (briefer) opening of the LES, the higher prestimulus basal pressure following devazepide pretreatment, and the greatly reduced hindstomach contraction evoked by CCK-8 following devazepide. These results point out the limitation of pressure measurements in large cavities: the measured pressures result from a combination of influences that cannot be distinguished by using manometry alone.

The onset of response to CCK did not differ between any of the traces, but the duration of response was not identical for all traces. Although no consistent pattern existed between response durations of the sphincters and those of antrum, corpus, or intragastric pressures, a reliable relationship existed between the sphincters themselves. Specifically, LES consistently closed before the contraction of pylorus had ended, and the difference in duration of response was statistically significant (P < 0.001). Typical examples of the temporal relationship between LES and pylorus are shown in Fig. 3. Although previously published reports have demonstrated contraction of pylorus and relaxation of LES in response to CCK separately (2, 3, 7, 14), we are aware of no prior studies tracking both simultaneously. Some methodological questions regarding identification of the point of effective closure in the motion traces

remain, and so these conclusions will have to be further substantiated by using concomitant manometric approaches. However, this coordination of LES and pylorus makes a great deal of physiological sense. If both sphincters were open simultaneously, it would be difficult if not impossible for the stomach to control the direction of flow of material through the gastroduodenal and gastroesophageal junctions. This observation may perhaps shed light on the source of reflux pathologies in some patients. Gastroesophageal reflux has been defined as a "chronic disorder related to the retrograde flow of gastroduodenal contents into the esophagus and/or adjacent" (16). Our work suggests that proper coordination of LES and pylorus ensures that the sphincters are not simultaneously open and



thus that improper coordination of the activity of the two sphincters could be responsible for the occurrence of reflux of gastroduodenal and gastroesophageal reflux in some patients. If this were the case, investigation of either pylorus or LES function alone in these patients might not reveal an obvious flaw. Instead, the problem might arise from dysregulation of the timing of action of each relative to the other.

History and methodological considerations

The ability to simultaneously measure motion directly at a variety of locations is very desirable in GI studies, in which the pattern of coordinated motion both within and between dis-

> FIGURE 2. Sonometric traces demonstrate that the cholecystokinin octapeptide (CCK-8) causes simultaneous contraction of pylorus (PYL) and antrum (ANT), opening of LES, and inhibition of prevailing rhythmic (3-6/min) contractions, concomitant with a reduction in intragastric pressure (IGP). The direction of change in circular corpus (CORci) varied between animals but was consistent and doserelated in a given animal. A: in this animal, antral motion was not measured, and instead both longitudinal corpus (CORIn) and circular corpus crystal pairs were used. B: in this animal, antral motion was measured rather than that in the longitudinal corpus.



FIGURE 3. Ultrasonometry traces (LES, PYL) demonstrating that LES returns to baseline levels (reclosure) before pylorus (reopening) following intravenous CCK-8 injection. Vertical hatched lines indicate onset of response (both LES and pylorus), recovery of prestimulus LES intracrystal distance (90% of pre-CCK-8 tonic level), and recovery of prestimulus pylorus intracrystal distance (90% of pre-CCK-8 tonic level).

tinct regions of the gut is of great interest. It is therefore unclear why it has taken so long for this technology to penetrate the field of GI physiology. Sonomicrometry has been used in the field of cardiovascular physiology since the mid-1950's (17), but we are aware of only a small number of peer-reviewed studies detailing its use in GI motility studies (12, 15), with none published in the last nine years. Possible reasons may be related to a combination of detailed technical issues. First, it is only in recent years that piezoelectric crystals capable of transmitting omnidirectionally have been available. Additionally, the sensitivity of receiving crystals has been improved as the technology has matured. These two developments have relieved markedly the difficulty of positioning crystals so that reliable signals can be obtained.

Another relevant technical issue is the occasional occurrence of "level shifts" or other transient forms of noise in the sonometric signal. Level shifts are changes in the apparent intracrystal difference due to failure to trigger (detect the arrival of the incoming wavefront) on the same peak in the arriving wavefront consistently and instead triggering on an adjacent peak. This results in a change in apparent distance, determined by the time between peaks in the wavefront (equivalent to one wavelength of the ultrasonic frequency) and the number of peaks missed. Level shifts have rapid onset and offsets and can last from between a single cycle to indefinitely. The frequency of occurrence of prolonged level shifts can be minimized by proper positioning of crystals, although it is difficult to eliminate the occasional presence of transient level shifts or other rapid artifacts resulting from noise-induced changes in triggering. For cardiovascular, respiratory, or skeletal muscle studies, where a number of event cycles can occur within a few seconds, the presence of such transients several times a minute may not be intolerably intrusive. However, for GI studies, in which epochs lasting dozens of minutes to hours are common, the presence of this type of artifact can make for unattractive traces and could confound data analysis. Fortunately, these artifacts have distinctive signatures, i.e., rates of rise or fall far greater than any physiological signal, and so may be recognized and removed by software routines employing simple heuristic algorithms (Fig. 4).

A more significant restriction is imposed by the necessity for an uninterrupted aqueous or soft tissue medium along the straight-line path between crystals. Interruption of the straight line path between crystals by, for example, an air bubble or gas in a cavity will disrupt the measurement due to the markedly different transit time for ultrasound in gaseous vs. aqueous media. Thus, in the case of GI studies, it is frequently superior to place crystals along the same side of a surface of the gut rather than directly apposed across the lumen. In this case, the sound travels between the crystals through the smooth muscle and/or the surrounding aqueous tissue fluid, thus allowing for consistent measurement. With these issues overcome, it becomes possible to use ultrasonomicrometry to address a wide range of important questions in GI physiology.

Relationship to other methods

The most common techniques for examining GI smooth muscle activity in animal studies are manometric methods, strain gauge measurements, and EMG. Each of these methods provides valuable information regarding motility, but each also has limitations. Ultrasonomicrometry provides advantages for the study of GI motility that complement each of these available methods.

EMG measures electrical potential within the muscle. Spike activity, which is superimposed on slower underlying changes in electrical potential, signals generation of contractile force within the muscle. But changes in contractile force may be associated with shortening of muscle or an increase in wall stress during stretching of muscle. Additionally, it is typically not possible to distinguish between activity in the longitudinal vs. circular muscle layers when recording EMG activity, nor is it possible to directly distinguish relaxation, except as a decrease in spike activity against a background of ongoing spike activity. EMG recording has been used extensively to examine propagation of muscle activity along the GI tract, including the original identification of the myoelectric migrating complex, or MMC (19), and a variety of other phenomena (18). In general, EMG has been the method of choice for investigating temporal relationships of muscle activity in adjacent regions by using arrays of EMG electrodes. However, although the relationship of electric activities to a variety of types of GI motion have been studied (5, 19, 20), discrepancies between electric events and contractions are not uncommon. For instance, in a distended colon, spike activities known to be associated with contraction in an empty colon are not associated with a detectable colonic motion (9).

Sonomicrometry is well suited to examining propagating movement using arrays of piezoelectric crystals. Crystals can be positioned so that circular and longitudinal motion can be detected simultaneously and distinguished from each other, and enclosed volume can be determined via three-dimensional reconstruction. Additionally, placement of the crystals for acute experiments involves less trauma to the tissue than does placement of EMG electrodes. Such trauma is probably not an important factor in the sturdier tissues of larger animals, but in more delicate structures such as mouse intestine or sphincters it may become a more important factor. Certainly, extensive manipulation of the pylorus is known to disrupt its function for extended periods thereafter. Most importantly, sonomicrometry can detect both shortening and lengthening of muscle, as well as phasic and tonic activity.

These traits also make sonomicrometry a useful complement to strain gauge measurements. Strain is defined as deformation resulting from application of a stress, and bare strain gauges signal deformation (as a change in electrical resistance) rather than applied force. However, in GI studies, strain gauges are typically encased in physically resistant media such as plastic, and it is this package that is sutured to the GI smooth muscle at several points. Since the package has some degree of stiffness, deformation of the enclosed strain gauge requires a force sufficient to flex the package. Thus in GI motility studies the strain gauge functions as a mixed force and motion transducer and is often referred to explicitly as a strain gauge-force transducer. Typically, strain gauge activity is quantified as area under the curve of the data trace or, in studies done before computerized data acquisition made this a trivial calculation, as motility index, a weighted sum of large, medium, and small peaks. Although the integrated straingauge activity provides a measure of the contraction/contractile force per unit time, the raw trace obtained does not provide a clear picture of underlying tissue movement, given the variety of patterns of distorting forces capable of giving rise to similar signals. In contrast to EMG, strain gauges with directional selectivity are typically used and thus provide some information about the axis of contraction. Strain gauge measurements are better suited for detecting changes in phasic activity than for detecting prolonged changes in tone, and detection of relaxation using strain gauges works best when investigating reduction of activity against a background of active contraction (6). One concern regarding the use of strain gauges is that the strain gauge package, which is anchored to the underlying tissue at several points, may cause disruption of normal motion, particularly in delicate tissues.

The strengths and weaknesses of manometric methods (simple manometry, sidehole manometry, and Dentsleeve manometry) are the approximate inverse of those of EMG and strain gauge methods. Manometry can detect both tonic and phasic activity, as well as both net contraction and net relaxation. However, manometric methods generally provide poor spatial resolution, except where the local luminal volume is relatively small. Also, the occurrence of common cavities resulting from sphincter opening can interfere with the ability to detect local changes in luminal circumference. The sensitivity of pressure measurements to local wall motion varies in different regions and depends on the volume of the surrounding lumen and its continuity to adjacent compartments. In a large lumen, it is possible to have a great deal of wall motion without appreciable changes in total luminal pressure or vol-



FIGURE 4. Signal processing sequence. *Top*: raw data trace (LES during basal period) containing "digital noise" transients. *Middle*: trace "deglitched" by a software script that recognizes and removes transient digital noise and level shifts. *Bottom*: smoothing applied to deglitched trace to remove respiratory rhythm.

ume (11), as long as contractions in one region are balanced by relaxations in another. Interpretation of manometric data must take into account these complicating factors. For example, due to the reduced sensitivity of manometric methods in antrum and duodenum relative to pylorus, the question of whether isolated pyloric pressure waves (IPPWs) reflect contraction restricted to the pylorus was for some time controversial. In an attempt to resolve the issue, Edelbroek et al. (8) used a combination of strain gauges, EMG, and combined sidehole and sleeve manometry in the antrum, pylorus, and duodenum of dogs and found that ~25% of the IPPWs detected manometrically were accompanied by electrical spike activity in the distal antrum or proximal duodenum but concluded that IPPWs did indeed primarily reflect contraction limited to the pyloric sphincter.

Sonomicrometry, used in combination with EMG, force tranducers, or manometric measurements, will allow investigation of the relationship between muscle activity, force generation, pressure changes, and motion in a wide variety of preparations and physiological conditions. Sonomicrometry should prove particularly useful in combination with manometric methods since the movement of luminal contents is dependent on a combination of peristaltic motion and pressure gradients developed as a result of patterns of muscle contraction and relaxation (11) and because manometric methods currently provide the best indication of sphincter patency.

In summary, sonomicrometry provides a number of advantages for the study of motility. First and foremost, it provides a direct means of detecting motion rather than concomitants of motion. It combines the high spatial resolution of EMG and strain gauge measurements with the capacity to detect either stretching or contraction, to track both and tonic and phasic components of motion, and to distinguish circular and longitudinal axes of motion. It is suitable for use in small animals and on small structures, such as sphincters, and the placement of crystals on the serosal surface of GI tissues by using cyanoacrylate glue for acute experiments is minimally invasive compared with the methods required to affix EMG electrodes or strain gauges. Sonomicrometry can be used in fed or fasted animals, and the ease with which crystals can be placed at multiple locations makes it suitable for studying the organization of activity across large expanses of the GI tract, as for example occurs in gastrocolic reflexes. Sonometric signals have a direct physical interpretation and can also be used to perform three-dimensional reconstructions, thus allowing the measurement of enclosed volume and, in combination with simultaneous pressure measurements, of compliance. A great deal of work will be required to develop appropriate analytical techniques to analyze sonomicrometric data in the GI field and in particular to correlate motion signals to functional processes such as effective opening or closing of sphincters (determined using combined Dentsleeve/sidehole manometry), propulsive or retropulsive activity, mixing, and so on. But this work should yield a wealth of heretofore unavailable insight, and in the coming years sonomicrometry is likely to become a fundamental tool in the study of the physiology of the GI tract.

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