Evaluation of multiple-point measurement of sphincter of Oddi motility in the Australian brush-tailed possum

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Craig, A. G., T. I. Omari, G. T. P. Saccone, J. Toouli, and J. Dent. Evaluation of multiple-point measurement of sphincter of Oddi motility in the Australian brush-tailed possum. Am J Physiol Gastrointest Liver Physiol 279: G837–G843, 2000.—Manometric assembly diameter is a major limitation on the number of perfused manometric recording points for recordings from the sphincter of Oddi (SO). We evaluated novel polyimide manometric assemblies whereby four recording channels were incorporated in an overall assembly diameter of 0.8 mm. Over the very low range of perfusion rates tested (0.005–0.04 ml/min), the assemblies had pressure offsets attributable to water perfusion from 2 to 23 mmHg and pressure rise rates from 20 to 163 mmHg/s. In six anesthetized Australian brush-tailed possums, manometric recordings from the SO showed a significant reduction in the recorded peak amplitude of pressure waves with perfusion rates below 0.02 ml/min. The pressure profile of the sphincter was found to be asymmetric, and phasic wave propagation patterns were complex (antegrade 35.6%, “mixed” 64.4%). In conclusion, accurate multipoint SO manometry in the possum can be performed with micromanometric assemblies at very low perfusion rates to give a more complete understanding of SO mechanics. These methods are also potentially applicable to perfusion manometry in other small laboratory animals such as mice.

LOW-COMPLIANCE PERFUSION MANOMETRY is a well-established technique for recording intraluminal pressures within the gastrointestinal tract (9) and has been widely used to assess sphincter of Oddi (SO) motility in humans (2) and animals (3). In the Australian brush-tailed possum, the measurement of SO motor function with perfusion manometry has been limited by the small size of the SO lumen and the necessity of minimizing the volume of water infused to prevent nonphysiological levels of distension of the sphincter. The standard approach in this species has been to use single-lumen manometric catheters with an outer diameter (OD) of 0.6–1.0 mm that are perfused at flow rates of 0.15–0.2 ml/min. In other short regions of the gastrointestinal tract that play a significant role in flow control, such as the lower esophageal sphincter and pylorus, the use of an array of closely spaced point manometric sensors has greatly enhanced the spatial and temporal resolution of intraluminal pressure events, thus giving much improved insights into mechanisms relevant to control of movement of intraluminal contents (11). To date, the absence of suitably miniaturized multiple-lumen manometric assemblies has prevented similar evaluations of SO pressures in both humans and animals.

Recent evaluations of micromanometric recording techniques have shown that reduction of manometric luminal diameter enables accurate manometric recording of gastrointestinal pressure waves at significantly reduced rates of manometric perfusion because of the marked reduction in compliance consequent on reduction of luminal diameter (1, 5). These evaluations have important implications for biliary tract manometry, which currently uses rates of manometric infusion that are high enough to cause significant problems with the rate of water delivery to the very small space of the SO. Existing technical insights suggest that with technical refinement, manometric perfusion rates could be reduced by up to a factor of 7. Furthermore, a reduced diameter of recording lumina means that a greater number of these lumina can be incorporated within a standard assembly cross-section. The aim of this study was to evaluate the dynamic performance of four-

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men manometric assemblies with recording lumina of 0.25 mm and an OD <1 mm over a range of much lower manometric perfusion rates than have yet been used in this measurement setting. These assemblies were then used for multiple-point measurement of SO motility in the Australian brush-tailed possum.

**METHODS**

**Manometric Perfusion System**

The manometric tubing and assemblies were perfused with a low-compliance manometric perfusion pump (Dentsleeve, Adelaide, South Australia, Australia). For assessment of dynamic performance of manometric materials and manometric assemblies, specific hydraulic resistors (Dentsleeve, Wayville, South Australia, Australia) were used to produce accurate perfusion flow rates of 0.005, 0.01, 0.02, and 0.04 ml/min at a constant driving pressure of 100 kPa. For in vivo studies, a single resistor type was used and the above perfusion rates were achieved by setting the driving pressure of the perfusion pump to 25, 50, 100, and 200 kPa, respectively. Also, for the in vivo studies, the hydraulic resistors, which were made from lengths of tubing of very small inner diameter (ID), were used to connect the perfusion pump to the transducers, which were positioned as close as possible to the animal. By this means, the overall length required for the manometric assembly was reduced substantially (Fig. 1). The distance from the manometric assembly side hole to the transducer is a crucial determinant of manometric assembly compliance and its resistance to perfusion (9).

**Manometric Assemblies**

Purpose-built four-lumen micromanometric assemblies were constructed with 10-cm individual lengths of single-lumen polyimide tubing (0.25-mm ID × 0.30-mm OD; Micro-Biomedical Tubing, Cartersville, GA), which were glued together to form the assembly (Fig. 2A). The polyimide tubing was chosen because preliminary testing showed it to have a very low compliance despite its very thin wall relative to its ID. This thin wall minimized the overall diameter of the built-up assembly. The polyimide tubing was interfaced to 30-cm lengths of silicone rubber tubing (0.40-mm ID × 2.20-mm OD; Dentsleeve, Wayville) to give an overall assembly length of 40 cm. Two assemblies were constructed. **Assembly 1** (Fig. 2B) incorporated four side holes located radially at the same position 2 mm from the assembly tip. This assembly was used to evaluate the radial pressure profile of the SO. **Assembly 2** (Fig. 2C) incorporated four side holes located at 4-mm intervals beginning 2 mm from the assembly tip.

**Assessment of Dynamic Performance of Manometric Assemblies and “Manometric System”**

A “testing assembly” with the same luminal dimensions and length as **assemblies 1 and 2** was used for bench testing of pressure rise rates and pressure offset with perfusion. This assembly did not have side holes; instead, its manometric channel was open at the tip. It was flushed with CO₂ to minimize bubble entrapment and then connected to the perfusion system with a low-compliance silicone rubber Luer that eliminated the dead space between the resistor and the transducer Luer. The open end of the polyimide tube assembly was cannulated with a blunted 25-gauge needle that was then connected to a noncompliant high-pressure stopcock normally used for high-pressure liquid chromatography (Hamilton Valve, Reno, NV). Closure of the manometric lumen with the stopcock ensured reproducible, complete occlusion without generation of pressure shock waves. Recordings were made of the pressure rise rate after manometric channel occlusion over the range of 0–200 mmHg above the preocclusion baseline. Pressure rise rates (dP/dt) were calculated for the first 100 mmHg of pressure rise and are expressed in millimeters of mercury per second. The lumen was occluded four times, and the mean pressure rise rate was calculated. Manometric performance was evaluated at perfu-
sion rates of 0.005–0.04 ml/min with specific resistors for each perfusion rate at 100 kPa. After this, the assembly was detached from the transducer, and the compliance of the transducer itself and its connections was measured by attachment of the stopcock directly to the transducer Luer hub. The fluid path was again occluded four times, and the pressure rise rate was calculated. Before the bench test, the net volume of perfusate produced by the hydraulic resistors was accurately determined gravimetrically with an electronic scale. For each resistor, the measured perfusion rate was within ±5% of the specified flow.

**In Vivo Studies**

**Animal preparation.** Studies were performed on six Australian brush-tailed possums (Trichosurus vulpecula) of either sex, weighing 1.9–3.3 kg, as previously described (8). Briefly, each animal was fasted overnight before anesthetic induction with intramuscular ketamine hydrochloride (20 mg/kg; Ketamil, Troy Laboratories, Smithfield, New South Wales, Australia) and xylazine (5 mg/kg; Ilium Xylazil, Troy Laboratories). Anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (20–50 mg·kg\(^{-1}\)·h\(^{-1}\); Nembutal, Rhone Menieux Australia, Pinkenba, Queensland, Australia). A tracheostomy and tracheal intubation allowed mechanical ventilation. The femoral artery and vein were cannulated to monitor blood pressure and provide venous access, respectively. A midline incision gave access to the abdominal cavity. The manometry assembly was positioned within the SO via an incision in the mid-common bile duct. A cannula was inserted in the proximal common bile duct to allow diversion of bile to the duodenum distal to the SO. A duodenal segment aboral to the SO was isolated, and an external drainage catheter was inserted into the segment to allow effective drainage of duodenal secretions. A 30-min period of equilibration was allowed before studies commenced.

**Evaluation of effect of perfusion rate on pressure wave recordings and radial pressure profile.** This was evaluated in three animals with assembly 1. All side holes were perfused at the same perfusion rate, and this perfusion rate was simultaneously varied on all lumina from 0.005 to 0.04 ml/min by altering pump driving pressure as described in **Manometric Perfusion System**. With the assembly perfused at 0.02 ml·min\(^{-1}\)·lumen\(^{-1}\), the most active region of the SO

**Fig. 2.** A: cross-section of the polyimide-epoxy resin assembly shaft tip showing details of the 4 lumina. OD, outer diameter; ID, inner diameter. B: **assembly 1** is schematically represented as being within the sphincter of Oddi (SO). The radially orientated side holes can be seen; an example of a recording made with this assembly is included on the right. C: the schematic depiction of **assembly 2** demonstrates the chain of side holes used to assess pressure wave propagation; an example of a recording is included on the right.
was detected by stepwise pull-through. The assembly was then secured so that its side holes were held in this active region with a ligature around the common bile duct 4 cm from the SO. The effect of perfusion rate on pressure wave recordings was then determined after a 30-min equilibration period, the perfusion rate being increased first to 0.04 ml/min and then altered in a stepwise fashion through 0.02, 0.01, 0.005, 0.01, and 0.02 ml/min back to 0.04 ml/min (see above). With each step of the driving pressure, at least five pressure waves were recorded before the flow rate was changed. Recorded pressure waves were analyzed to determine the effect of perfusion rate on pressure wave characteristics. One hundred and twenty pressure waves were analyzed at each manometric perfusion rate. The radial pattern of pressure at that point was then assessed at a perfusion rate of 0.02 ml/min by evaluating five sequential pressure waves at each of the four recording points.

**Pressure wave propagation.** Propagation of SO pressure wave sequences was evaluated in a further three animals with assembly 2. The assembly was positioned with the side hole array cradling the sphincter zone with the tip located at the duodenal-SO junction. Again, to maintain the recording position, the assembly was tied to the common bile duct at its point of insertion before the equilibration period. The assembly was perfused at 0.02 ml/min. After the equilibration period, 30 pressure wave sequences were analyzed for wave propagation characteristics.

**Analysis of manometric tracings.** Manometric recordings were analyzed directly from the display of the tracing using tools provided by the recording software. The peak amplitude, slope of the upstroke, and area under the curve (AUC) were determined for each pressure wave analyzed. Peak amplitude was defined as the highest pressure achieved above baseline. The slope of the pressure wave was defined as the maximum rate of pressure rise during the upstroke, as measured from the point of onset. AUC was measured for the duration of the pressure wave. To determine whether there was radial symmetry of the pressure profile, in each animal the coefficient of variation in recorded peak amplitude of each simultaneously recorded pressure wave was determined between all side holes.

Criteria for determining SO phasic wave propagation are not well defined in the literature. Judgments have been made somewhat subjectively by assessing the onset of the upstroke of phasic waves in relation to other recorded phasic waves, often by simply drawing a line between the points of onset. Spatial organization has then usually been described as propagating in the antegrade (toward duodenum) and retrograde directions, or the SO segment has been judged to contract simultaneously. The number of recording points is usually two or three, and often just the most proximal and distal recording points are analyzed (15). To our knowledge, SO phasic wave propagation has never been described for more than three recording points in the possum or other species, including humans. For this study, the onset of the pressure wave was taken as the time at which the tangent of the major upstroke of the pressure wave bisected the baseline pressure as described for evaluations of space-time patterns of pressure waves in the antroduodenal region (11). Antegrade propagation was defined as the commencement of pressure wave sequences in the most proximal portion of the SO, adjacent to the common bile duct and progressing sequentially with an interval of at least 100 ms between all side holes toward the distal side hole adjacent to the duodenum. Retrograde propagation was defined when the pressure wave started in the distal side hole, progressing sequentially in each side hole toward the proximal side hole at least 100 ms relative to the next side hole toward the common bile duct. Simultaneous occurrence was defined when all pressure waves recorded from adjacent SO side holes started within 100 ms of each other. If the propagation did not fall into any of the above definitions, it was characterized as mixed.

**Data Aquisition, Analysis, and Statistics**

Data were acquired and analyzed on a computer recorder (MacLab, AD Instruments, Castle Hill, New South Wales, Australia). Data are expressed as mean values ± SE unless otherwise stated. Grouped-data comparisons between different flow rates for pressure wave characteristics were performed using ANOVA. A P value of <0.05 was judged to be significant.

**RESULTS**

**Dynamic Performance Testing of Manometric Assemblies and Manometric System**

The rate of pressure rise recorded by the transducers without assemblies attached was a measure of the manometric system compliance other than that due to the manometric assembly itself. This rise rate increased linearly according to perfusion rates and ranged from 68 ± 16 to 542 ± 119 mmHg/s at perfusion rates of 0.005–0.04 ml/min (Fig. 3). With the assemblies attached to the system at perfusion rates of 0.005–0.04 ml/min, baseline offsets ranged from 2 to 23 mmHg and rise rates from 20 ± 5 to 163 ± 39 mmHg/s.

**In Vivo Studies**

**Evaluation of effect of perfusion rate on pressure wave recordings and radial pressure profile.** By comparison with perfusion rates of 0.02 and 0.04 ml/min, recorded pressure wave amplitude and slope were significantly lower when the manometric perfusion rate was reduced to <0.02 ml/min. The amplitudes and slopes of recorded pressure waves were similar for perfusion rates of 0.02 and 0.04 ml/min (Fig. 4). The mean maximum slope at 0.04 ml/min was 38.2 mmHg/s, which is well below the maximum rise rate of 163 mmHg/s at this perfusion rate, as determined by the bench testing. The mean maximum slope of the highest 50% of phasic waves for this perfusion rate was 49.4 mmHg/s, again well below the maximum rise rate.

![Fig. 3. The effect of manometric perfusion rate on pressure rise rate of the manometric assembly (solid line). The graph demonstrates the linear relationship. The dashed line shows the pressure rise rate of the transducer alone for comparison.](http://ajpgi.physiology.org/)
The peak pressure, maximum slope, and AUC of the pressure waves recorded by each channel positioned at the same axial level varied little from wave to wave; however, the circumferential pressure profile of the SO at the point of measurement was asymmetric (Fig. 5). For each animal, the mean coefficients of variation between side holes for peak amplitude, slope, and AUC were 26.9%, 24.4%, and 27.5%, respectively; by contrast, pressure wave onset was simultaneous on all channels.

Pressure wave propagation. Of the 90 propagated pressure wave sequences analyzed, 58 (64.4%) were mixed and 32 (35.6%) were antegrade for the four recording points. No completely simultaneous or retrograde sequences were recorded.

DISCUSSION

We have evaluated the performance of novel manometric assemblies for multiple-sensor measurement of SO motility in the Australian brush-tailed possum. Our recordings indicate that the pressure profile of the possum SO is radially asymmetric and that almost two-thirds of pressure wave sequences are propagated in a complex pattern, a purely antegrade peristalsis pattern being seen in the other sequences.

Dynamic performance testing of these assemblies indicates that pressure rise rates of 80–160 mmHg/s could be achieved at manometric perfusion rates of 0.02–0.04 ml/min, consistent with the low compliance of the polyimide tubing and the short length of the manometric assembly. Given the small size of the possum SO, it was not possible to use an intraluminal transducer to record the true pressure within the possum SO. The only alternative was to “overperfuse” and consider this as the gold standard, provided that the pressure rise rate achieved was substantially in excess of that of the recorded pressure waves. If the data obtained at a perfusion rate of 0.04 ml/min are used as the gold standard, a perfusion rate of 0.02 ml/min appears to be optimal for in vivo measurement of possum SO pressure waves, given that the recorded pressure wave characteristics were only significantly different from those obtained at a perfusion rate of 0.04 ml/min at perfusion rates below this level. At a perfusion rate of 0.02 ml/min in our possum SO model, this flow rate is one-seventh that of the “standard” rate of manometric perfusion used for SO manometry (0.15 ml/min). Thus, even when all four lumina are in use, the total volume of perfusate delivered to the SO (0.08 ml/min) is one-half that of a standard single-lumen assembly. The importance of using the lowest possible perfusion flow rate is emphasized by the finding that...
balloon distension of the American opossum SO has been shown to alter the phasic frequency (13).

In a previous study, multiple-point manometric recordings were made from the American opossum SO with an extrusion that had an OD of 1 mm and three lumina of 0.3 mm (13). These studies were hampered by high pressure offsets due to the relatively high flow rate that was used (0.1 ml/min). High pressure offsets can lead to fluctuations in the baseline recordings from minor variations in the driving pressure of the perfusion system and so are best avoided when studying sphincteric regions. Baseline offset can be reduced by lowering the perfusion rate and/or shortening overall assembly length. In the current study, we were able to reduce assembly offset to <25 mmHg by using especially low-flow “uncoiled” hydraulic resistors that 1) reduced the rate of manometric perfusion to more appropriate levels (0.005–0.04 ml/min) and 2) enabled the pressure transducers to be brought as close as possible to the recording site, therefore reducing the total length of the manometric assembly needed.

Our data show that the possum SO radial pressure profile has substantial asymmetry. This has also been found to be the case in dogs (12) and has been reported in abstract form in humans (10). The asymmetry of the possum SO is most likely to be explained by the absence of circular muscle fibers in the fibromuscular septum between the biliary and pancreatic segments of the sphincter (6). Unlike the pressure wave peak amplitude, the timing of pressure wave onset was not affected by the radial pressure profile. Hence, when we evaluated the propagation of pressure wave sequences, we examined the timing of the onset of pressure waves rather than the timing of the peaks. At least in the human esophagus, the accurate recognition of the timing of the onset of a pressure wave upstroke is of more relevance functionally than the time that peak amplitude is reached, or its magnitude, because pressure waves of different peak amplitudes are equally effective in facilitating bolus transit, provided they both reach a threshold amplitude and have an aborally sequential timing of onset (7). It is therefore reasonable to conclude that manometric recordings made at very low perfusion rates with rise rates that may fail to record peak amplitude accurately are of value in the assessment of motor function, provided that a threshold pressure is measured accurately. As yet, there is insufficient knowledge about SO mechanics to identify this threshold pressure in the SO.

The direction of phasic wave propagation has not been reported previously in the possum. Visual inspection of the external surface of the sphincter reveals apparent antegrade phasic wave propagation. In contrast, our data indicate that many pressure wave sequences are spatially complex in their timing of onset, with the middle two recording points showing the phasic wave onset time being simultaneous or even having a partially retrograde pattern. It is of note that we found that the propagation pattern of pressure waves in the most proximal (i.e., adjacent to the common bile duct) portion of the SO was always antegrade. The functional correlation of this complex propagation behavior is undetermined, but multipoint manometry will be important in unraveling whether the possum SO acts as a “pump” or a “resistor” or as both under different physiological conditions. Three-point manometric recordings from the human SO also show that the propagation patterns of luminal pressure waves are complex (14, 15), supporting the use of the Australian brush-tailed possum as a model for SO motility research.

Another issue that this study addressed was the limits of very low-perfusion-rate manometry as assessed by the rise rate of the transducer and its connectors alone. Our results demonstrated that the transducer does impose limits on the rise rate that can be achieved, presumably related to very minor material compliance within the transducer and of sealing surfaces of hydraulic connectors to the transducer, and these limitations must be considered when using very low perfusion rates. It is important to consider the effect of the manometric assembly itself, which introduces another source of compliance. Compliance attributable to the assembly is minimized by reducing the length of the manometric assembly, by using minimally compliant materials for manufacture of manometric assemblies, by using low-compliance connectors with minimal potential for airspace trapping (e.g., silicone rubber Luers), and by removal of microbubbles from the manometric lines by CO2 flushing. However, it should be recognized that perfusion rates that are so low that they do not faithfully signal the amplitude of pressure waves (e.g., 0.005 ml/min) may still be suitable for recordings where the timing of the pressure wave onset is the variable of primary importance. Also, such low perfusion rates are acceptable when recordings are being made from regions where low-amplitude pressure waves with modest rise rates are present.

This study demonstrates the feasibility of miniaturization of gastrointestinal manometric assemblies to a level not previously attempted. Assemblies of this overall diameter could be used for multilumen intraluminal manometry not only in the biliary tree but also in the gastrointestinal tract of small laboratory animals. In particular, these small assemblies with very low perfusion rates could be used for measurement of gut motility in mice, an important application given the availability of knockout mice, which have absence of specific parts of the gastrointestinal motor control mechanisms and can serve as models for abnormalities of gastrointestinal motor function (16). At the moment, there is a lack of techniques that are sufficiently miniaturized to make a direct assessment of spatial patterns of motility in such small animals.

Human perendoscopic SO manometry can be improved by incorporating some of the approaches used in this study. In particular, some very low-compliance materials such as polyimide can be extruded to give multiple lumina separated by very thin partitions. Consequently, it should be possible to substantially increase the number of recording lumina without increasing the overall outer diameter of the
assembly. Importantly, with well-tuned materials and techniques, perfusion rates can be reduced so that the overall infusion volume will not increase with the use of more lumina and may even be reduced. Reduced perfusion rates are associated with a lower incidence of post-endoscopic retrograde cholangiopancreatography pancreatitis (4), a significant clinical problem of this widely used diagnostic procedure. The manometric assemblies used in this study were essentially a concept test. The very thin walls of the polyimide tubing resulted in a remarkably small assembly for the number and diameter of the lumina, but the assemblies tested were very difficult to build and brittle because of the use of epoxy resin as an adhesive and filler around the lumina. Such assemblies are of limited practicality in any setting but are clearly not suitable for human SO manometry, which requires passage of the assembly down an endoscope and manipulation of the assembly tip. We are now pursuing the definitive option of a multilumen extrusion with a cross-section designed around that reported in this present study.

In conclusion, accurate multiple perfused side hole measurement of SO pressures has been made possible in the Australian brush-tailed possum by the use of polyimide micromanometric assemblies. The outcomes have been enhanced by reduction in the distance from recording points to the transducer. This approach has allowed substantially lower rates of manometric perfusion than those required with currently used techniques. These principles can be readily applied to other animal models of SO motility and human perendoscopic SO manometry as well as for intraluminal manometry in small laboratory animals.

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