Viscoelastic properties of the human colon

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Bharucha, Adil E., Rolf D. Hubmayr, Irene J. Ferber, and Alan R. Zinsmeister. Viscoelastic properties of the human colon. Am J Physiol Gastrointest Liver Physiol 281: G459–G466, 2001.—Our objectives were to characterize colonic viscoelastic properties of the human descending colon by assessing pressure-volume (P-V) relationships during barostatic balloon distension. In 16 healthy subjects, a balloon was inflated to 44 mmHg and then deflated to 0 mmHg in 4-mmHg steps at 10, 30, and 60 ml/min, allowing volume fluctuations to stabilize at each pressure increment. Thereafter, these “quasi-static” P-V curves were compared with “dynamic” distensions to 300 ml, at 1 and 10 ml/s, before and after intravenous atropine in another five subjects. During quasi-static curves, balloon volume stabilized at each pressure increment. Quasi-static P-V curves were reproducible within individuals and approximated to a power exponential function and revealed hysteresis, indicative of viscoelasticity. Body mass index influenced quasi-static P-V curves during inflation but not during deflation. The colon was less compliant during dynamic distensions at 10 ml/s than during quasi-static distensions. Atropine increased quasi-static compliance and attenuated differences between quasi-static and rapid distensions. We conclude that colonic viscoelastic properties can be assessed by quasi-static P-V curves. Rapid colonic distension activated neural reflexes, thereby reducing colonic compliance compared with quasi-static distensions.

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METHODS

After approval by the Institutional Review Board at the Mayo Clinic, 21 healthy subjects were recruited for these studies by public advertisement. All volunteers signed informed consent forms before participation. In part A, the intrasubject reproducibility of quasi-static colonic P-V relationships on a given day was assessed in 16 healthy subjects. In part B, atropine was administered to assess the effect of muscarinic cholinergic blockade on P-V relationships in five healthy subjects.

Healthy Volunteers

Eight male and eight female volunteers (mean age 37 years, range 20–54 years) participated in part A. Three male and two female volunteers (mean age 36 years, range 25–46 years) participated in part B. A clinical interview and physical examination were performed to exclude significant cardiovascular, respiratory, neurological, psychiatric, or endocrine disease. No subjects had previously undergone abdominal surgery (other than appendectomy and/or cholecystectomy) or were taking medications with the exception of oral contraceptives. The previously validated screening questionnaires, the Bowel Disease Questionnaire (30) and the Hospital Anxiety and Depression Inventory (36), were used to exclude subjects with irritable bowel syndrome and significant anxiety and depression.

Colonic Motor Activity

Methods. After an overnight oral lavage with 2–5 l of polyethylene glycol 3350 and electrolyte solution (Golytely, Abbott Laboratories, Chicago, IL), a multilumen polyethylene balloon barostat-manometric assembly was positioned in the descending colon using flexible endoscopy and fluoroscopy (6). Premedication was not given. Colonic motor activity was quantified using an infinitely compliant 10-cm long balloon (Hefty Baggies, Mobil Chemical, Pittsford, NY) linked to an electronic rigid piston barostat (Engineering Department, Mayo Clinic, Rochester, MN) by a double-lumen tubing with a larger lumen (3.2-mm inner diameter) for balloon distension and a smaller lumen (2-mm diameter) for measuring pressure (31). The transmural pressure in the balloon, inflated up to 500 ml outside the colon, was 0 mmHg, i.e., the balloon was infinitely compliant in vivo. Intra-balloon volume and pressure were recorded continuously, and a pneumobelt was applied to the abdominal wall at the level of the lower costal margin to exclude artifact during movement and coughing.

Reproducibility of Quasi-Static Colonic P-V Relationships: Part A

Methods. Four quasi-static P-V curves were performed in randomized order in each subject with an equilibration period of 30 min between distensions (Fig. 1A). Each curve comprised a stepwise inflation from 0 to 44 mmHg, followed by a graded deflation from 44 to 0 mmHg in 4-mmHg steps (Fig. 1A, inset). These were performed at 10, 30, or 60 ml/s, in randomized order. At each pressure increment, balloon pressure was maintained for as long as it was necessary for fluctuations in balloon volume to stabilize, i.e., to achieve steady state. Steady state was defined a priori as a fluctuation in balloon volume of <10%, compared with the volume at the same pressure 30 s previously. When this threshold for balloon volume was reached, balloon pressure was increased by 4 mmHg during the inflation limb or reduced by 4 mmHg during the deflation limb. Balloon pressure was maintained for a maximum duration of 5 min for pressures between 0 and 24 mmHg and for 2.5 min for pressures between 28 and 44 mmHg.

Data analysis. Barostat balloon pressure and volumes were acquired at 10 Hz and averaged over consecutive 5-s epochs. All subsequent data analysis was conducted using the mean 5-s data values.

To evaluate the time required for balloon volume to reach steady state, we computed a five-point running mean volume that spanned observations over 25 s, i.e., a given (mean 5 s) value, two values before and two values afterward. The mean SD and coefficient of variation (CV, expressed as %) for these overlapping five-point running mean volumes were computed at each pressure step for each curve in each individual. The CV of the five-point running means was summarized within subjects by computing the simple mean over consecutive 30-s epochs starting at the onset of each pressure step, i.e., from 0 to 0.5 min, 0.5 to 1 min, 1 to 1.5 min, and 1.5 to 2 min. Thus there were a maximum of four epochs for a given pressure. The average CV was analyzed across subjects (see Statistical Analysis).

In a separate analysis, an “observed” quasi-static P-V relationship (or compliance curve) was obtained by plotting the mean balloon volume from the last 30 s at each step against the balloon pressure. Each compliance curve was summarized, as described previously (5, 6, 19), using a power exponential model for the proportionate volume (V/Vmax) as a function of reciprocal pressure (1/P), i.e.: \( V/V_{\text{max}} = r + \exp \left(-k \times (1/P)^b \right) \) where \( V \) is the actual volume, \( V_{\text{max}} \) is the maximum volume, and \( r \) is the observed value of proportional volume at the initial pressure point (usually 4 mmHg). The parameter \( b \) affects the overall shape of the “fitted” curve, whereas \( k \) is essentially the change in volume as a function of 1/P at any given point. The parameters \( k \) and \( b \) for each compliance curve (colonic and rectal, pre- and postdrug) were estimated using the NLIN procedure in the SAS software package (26). The estimated \( k \) and \( b \) for each subject were used to calculate the pressure corresponding to half-maximum volume. The pressure at the inflection point was defined as the pressure corresponding to the value of the highest second-order derivative for the fitted P-V curve plotted using the formula given above. The area between the infla-
tion and deflation limbs of the observed quasi-static curve represents hysteresis. Hysteresivity (q) normalizes this area by expressing it as a proportion of the total area contained within the rectangle enclosed by the P-V loop (13) (see Fig. 3).

Comparison of Static and Dynamic P-V Relationships:

Part B

Methods. The objective of these studies was to compare quasi-static P-V curves to P-V curves during rapid (10 ml/s) and slow distensions (1 ml/s) before and after muscarinic cholinergic blockade with atropine. For quasi-static curves in part B, balloon pressure was maintained for 2 min at each step for pressures between 0 and 24 mmHg and for 1 min at each step for pressures between 28 and 44 mmHg. Rapid and slow distensions were conducted by inflating the balloon from 0 to 300 ml at flow rates of 10 and 1 ml/s in randomized order (Fig. 1, B and C, respectively). In contrast to quasi-static P-V curves, intervening steps did not interrupt balloon inflation from the minimum to the maximum volumes. Thus the comparison between quasi-static and ramp distensions allowed us to assess the effect of altering the rate of distension on P-V relationships.

Data analysis. Balloon volume and pressure data were acquired at 10 Hz and averaged over consecutive 5-s epochs for the quasi-static P-V curves and over 1-s epochs for ramp distensions to 300 ml. The observed quasi-static curves were fit using the same nonlinear model described for part A. Thereafter, all six fitted curves (pre- and postatropine quasi-static, 1 and 10 ml/s) in each subject were normalized to the observed maximum balloon volume in that subject; for all subjects, this was the volume at a pressure of 44 mmHg during the postatropine quasi-static curve. Quasi-static and dynamic (ramp distension) curves were compared by averaging the pressure values within consecutive tenth percentile volume epochs, i.e., from 0 to 10%, 11 to 20%, and so on, through 90 to 100%, for each of the six P-V curves separately for each subject.

Atropine

Atropine was administered as an intravenous bolus at 15 μg/kg followed by an infusion at 1 μg·kg⁻¹·h⁻¹ for 45 min with a reduction to 0.33 μg·kg⁻¹·h⁻¹ thereafter. Although we did not measure plasma concentrations of atropine, this regimen was designed to achieve and maintain an estimated plasma concentration of atropine ranging between 7 and 9 μg/dl (4). Heart rate and blood pressure were monitored at periodic intervals throughout the study using a telemetric monitoring device (Propac, Protocol Systems, Beaverton, OR).

Experimental Design

All subjects were admitted to the General Clinical Research Center at St. Mary’s Hospital on the evening before the study for a screening electrocardiogram to exclude significant rhythm disturbances or ischemia, to obtain a plasma β-human chorionic gonadotropin pregnancy test for women of childbearing potential, and for a bowel preparation. Subjects drank 2–5 l of polyethylene glycol 3350 and electrolyte solution (OCL, Abbott Laboratories) until the fecal effluent became clear. Subjects were fasted overnight, and left-sided colonoscopy was used to place the barostat-manometric assembly without sedation. After a 30-min equilibration period, the barostat “operating pressure” was set (5, 6) and the experiment started.

Statistical Analysis

To ascertain the time required for P-V relationships to reach steady state, we summarized the average CV of running mean volumes as the median and interquartile range across subjects at each pressure step. The effects of rate and order of distension on quasi-static compliance curve parameters were analyzed using a repeated-measures model ANOVA, fitting terms for rate and order (of distensions). Similar, albeit separate, analyses were conducted for the compliance parameters k, β, inflection point pressure, pressure corresponding to half-maximum volume, minimum volume, maximum volume, and hysteresis. An interaction term (e.g., rate by order) was included in these analyses to assess whether the effect of infusion rate was modified by the order of distension. An unstructured variance-covariance matrix was used to accommodate within subject correlations. Differences in compliance curve parameters (part A) using data from the first 30 s vs. the last 30 s at each pressure were compared by parametric (paired t-test) or nonparametric tests (Sign test) using a two-sided alpha-level of 0.05 to assess statistical significance. The association between the estimated parameters characterizing the quasi-static P-V curves and body mass index (BMI) was assessed using Spearman’s rank correlation.

The differences in mean balloon pressures between quasi-static and dynamic curves were computed within each tenth percentile volume interval. These differences were compared
Table 1. Effect of rate and order of distension on quasi-static P-V relationships

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rate of Distension, m/s</th>
<th>Order of Distension, curve no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>30 (1st)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>15.1 ± 0.7</td>
<td>15.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(19)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>2.0 ± 0.1*</td>
<td>1.9 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
<td>(16)</td>
</tr>
<tr>
<td>$P_{\text{inflection point}}$, mmHg</td>
<td>7.9 ± 0.1</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(32)</td>
<td>(28)</td>
</tr>
<tr>
<td>$P_{\text{half}}$, mmHg</td>
<td>16.3 ± 0.7</td>
<td>16.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(17)</td>
</tr>
<tr>
<td>Hysteresis</td>
<td>0.2 ± 0.01</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(17)</td>
</tr>
</tbody>
</table>

Values are means ± SE. P-V, pressure-volume; $\kappa$, change in volume as a function of reciprocal pressure; $\beta$, overall shape of fitted curve; $P_{\text{inflection point}}$, pressure at the inflection point; $P_{\text{half}}$, pressure corresponding to half-maximum volume. *$P = 0.003$ for effect of rate of distension on $\beta$. Nos. in parentheses are coefficient of variation (%).

before and after atropine using a paired t-test. Data are expressed as means ± SE unless stated differently.

RESULTS

Quasi-Static P-V Relationships

When the pressure was increased in 4-mmHg steps, the balloon volume increased rapidly at first and gradually thereafter, reaching a plateau with superimposed phasic fluctuations (Fig. 2). The time taken for balloon volumes to reach a plateau, or to achieve steady state, was typically 2 min for pressures ≤16 mmHg and 1 min for pressures ≥20 mmHg. At these times, the variability in balloon volume (CV) was ≤10% for pressures ≤16 mmHg and ≤5% for pressures ≥20 mmHg.

Exponential Configuration of Overall Quasi-Static P-V Relationship

For each subject, the average balloon volume over the last 30 s at each step was plotted against the corresponding pressure, yielding the observed quasi-static P-V relationship (Fig. 3). The fitted curves based on the power exponential function closely approximated the observed quasi-static P-V curves (median $R^2 = 0.996$; range, 0.946–1.0). In most subjects, this curve generally exhibited an initial flat portion, a transition to a steeper segment at an inflection point (maximum acceleration point), and a second transition to a flat portion at higher pressures. The ascending and descending limbs of the quasi-static P-V relationship were not superimposable but circumscribed an area, indicating hysteresis (Fig. 3; Table 1); $\eta$ expresses the area of the hysteretic curve as a proportion of the entire area contained within the rectangle shown in Fig. 3.

Intraindividual Reproducibility of P-V Relationships

Reproducibility of P-V curves within subjects was considered relative to rate and order of distension. Visual inspection of quasi-static P-V curves indicated that quasi-static P-V relationships were reproducible in 13 of 16 subjects or reproducible only after an initial or “conditioning” distension in 3 of 16 subjects (Fig. 4). The rate of distension did not influence quasi-static P-V curves during inflation (Table 1) or deflation (data not shown). Though the rate of distension significantly affected $\kappa$, $\beta$, half-maximum inflection point pressure, pressure corresponding to half-maximum volume, or hysteresis (Table 1), the average maximum volume increased progressively during successive curves, reaching a plateau at the third or penultimate curve (Table 2). For any given P-V curve, the balloon volume at 0 mmHg was higher after deflation vs. before inflation (Table 2). However, the balloon volume declined during the equilibration period between successive curves. Therefore, the balloon volume before inflation at 0 mmHg was very similar for all four curves.

![Fig. 4. Effect of order of distension on quasi-static P-V relationships. A: in 13 of 16 individuals all 4 quasi-static relationships were reproducible. B: in 3 of 16 individuals, quasi-static curves were only reproducible after a conditioning distension was performed.](http://ajpgi.physiology.org)
There was a significant correlation between body habitus, expressed as the BMI, and configuration of the compliance curve. As depicted in Fig. 5, the volume change accompanying balloon inflation in stepwise pressure increments up to 16 mmHg was influenced by the BMI, i.e., the volume change during a 4-mmHg stepwise increment was higher for lean subjects compared with obese subjects. Consequently, for the second distension at 30 ml/s, the BMI was directly associated with the maximum volume and inversely associated with the minimum volume (Fig. 5). A similar association between BMI and compliance parameters during the inflation limb was noted during other P-V curves (data not shown). These findings imply that as the BMI increased, the inflection point was shifted to the right, whereas the maximum volume was lower, i.e., the colon was “less compliant.”

To ascertain the effects of prolonging distension beyond 30 s at each pressure, we compared P-V curves by using the average volume during the first 30 s to those obtained during the last 30 s at all pressures. Figure 6 demonstrates that the correlation between estimated parameters of the compliance curve using volumes during the first and the last 30 s at each pressure is excellent. Nonetheless, some subjects exhibited small differences in parameters of the compliance curve estimated using volumes from the first 30 s vs. the last 30 s (Fig. 6).

**Effect of Atropine on Quasi-Static P-V Curves**

Atropine markedly reduced phasic activity recorded by manometric sensors in the descending colon (Fig. 7) and increased colonic compliance, shifting the overall P-V relationship to the left (Fig. 8); the inflection point occurred at a lower pressure (7.96 ± 1.19 and 5.1 ± 0.71 mmHg for predrug vs. atropine, respectively; P < 0.01). Atropine increased balloon volume during inflation to a greater extent than during deflation (P < 0.05), reducing η by ~50% from 0.16 ± 0.03 (predrug) to 0.08 ± 0.01 (postdrug).

**Comparison of Quasi-Static to Dynamic P-V Curves**

Figure 9 compares quasi-static P-V curves with ramp distensions in which the balloon was inflated to 300 ml at 1 and 10 ml/s. The quasi-static distensions from 0 to 44 mmHg took 18 min whereas the ramp distensions to 300 ml at 1 and 10 ml/s took 5 min and 30 s, respectively. Before atropine, balloon volumes and therefore compliances were lower at comparable pressures in the mid high-pressure range during rapid distension at 10 ml/s than during quasi-static P-V curve. Atropine attenuated the differences between quasi-static and 10 ml/s distensions.

**DISCUSSION**

We have validated a technique to assess quasi-static colonic P-V curves in the human descending colon and characterized the viscoelastic properties of the human colon. The key factor is that by waiting long enough for fluctuations in balloon volume to subside at each pressure increment, the effects of distension-induced neural activation on P-V curves could be minimized. Colonic P-V relationships approximated to a power exponential function and were reproducible within subjects, although a conditioning distension was required in some instances. Colonic compliance was lower during rapid than quasi-static distension; we believe this was because rapid distension evoked neural contractile reflexes. Indeed, these appeared to be attenuated by atropine. We believe our methodology was sound both in experimental design and because we avoided potential limitations in assessing viscoelastic
properties by using a highly compliant, polyethylene balloon connected by a double-lumen tubing with a rigid cylinder barostat (2, 17, 32). These observations are relevant to the physiology of the human colon and to methods being used to assess its pathophysiology.

The practical implications of our study are as follows. A conditioning distension should preferably be performed in studies of viscoelasticity and sensation (16). Thereafter, balloon pressure should be increased gradually from 0 to 44 mmHg, maintaining pressure for 2 min at each step for pressures between 0 and 16 mmHg and 1 min for pressures ≥20 mmHg when assessing colonic P-V curves in humans. Quasi-static P-V curves plotted by using average balloon volume during the last 30 s at each pressure step, i.e., after volume has stabilized, are reproducible within individuals; however, curve shape, particularly the inflection point, is different if pressure is maintained for only 30 s at each pressure. A comparison of quasi-static curves pre- and postatropine and dynamic curves provides an approach to dissecting the various contributors of colonic biomechanical properties, i.e., passive, active tonic, and active reflex contractile properties. Thus differences between quasi-static and dynamic curves probably reflect reflex responses to distension, whereas a comparison of quasi-static curves before and after atropine enables separation of muscle tone from passive properties.

Fig. 6. Compliance curve parameters estimated from balloon volumes at first 30 s (y-axis) are correlated but not identical to parameters calculated by using balloon volume during last 30 s (x-axis) at each step. Data pertain to inflation limb for second distension at 30 ml/s. A: pressure at inflection point. B: the change in volume as a function of reciprocal pressure (κ). C: the overall shape of the fitted curve (β). D: hysteresis.

Fig. 7. Effect of atropine on colonic motor activity. Atropine markedly reduced phasic pressure activity recorded by manometric sensors and increased balloon volume, both at constant pressure and during P-V curves.

Fig. 8. Effect of atropine on quasi-static P-V relationships in a single subject. Atropine increased the intraballoon volume at any given pressure and reduced η. **p < 0.05.
Conditioning distensions are used before mechanical testing in vitro (15) and also enhance the reproducibility of measuring tone and sensory thresholds in the rectum (16) and stomach (1) in humans. From a practical aspect, conditioning distensions are essential particularly if the objective is to compare the effects of placebo to a pharmacological agent on P-V curves in the same individual in 1 day. In the guinea pig jejunum, the effects of a conditioning distension appear to be predominantly attributable to maximum volume during distension (14). The maximum balloon volume at our highest imposed pressure increased during the first three P-V curves but plateaued thereafter, whereas the minimum balloon volume before inflation was similar prior to all four P-V curves. This suggests that substantial plastic or permanent distension did not occur during the slow distension paradigm we employed.

Overall, quasi-static colonic P-V curves approximated closely to a power exponential function defined by the parameters $k$ and $b$ (5, 6). The pressure corresponding to half-maximum volume, a useful summary parameter for the entire compliance curve, was derived from $k$, $b$, and the ratio of minimum to maximum volume, and was inversely correlated to compliance. The area between the P-V curve during inflation and deflation limbs indicates hysteresis, which, in contrast to elastance or compliance, reflects the work done and energy dissipated on the system during inflation that is not recoverable during deflation (20). The parameter $\eta$ normalizes hysteresis to the area of a rectangle encompassing the entire P-V curve. Comparing our data with results in other organ systems, the value for $\eta$ in the pretropine curves (i.e., $\sim 0.2$) was remarkably similar to that recorded in pulmonary tissue from a number of species (13). Similarly, the presence of hysteresis during quasi-static distensions was consistent with a plastic or rate-independent process as has been demonstrated in lung tissue (13). Lastly, atropine reduced $\eta$ during colonic P-V curves, consistent with the inverse observation that activation of the contractile machinery increases hysteresis in pulmonary tissue (11, 18).

The association between body habitus, expressed as the BMI, and the configuration of P-V curves during inflation has not been observed in previous studies (2, 15, 33). Thus BMI may be an important covariate in future studies assessing colonic P-V relationships. The association between BMI and compliance may be mediated by variations in intra-abdominal pressure, which is higher in obese subjects, particularly during straining (21, 27). Perhaps the higher intra-abdominal pressures that were associated with higher BMI indicate a resistance to expansion of the colonic balloon during inflation, shifting the inflection point to the right and reducing maximum volume at the highest imposed pressure. Potential solutions to avoid the confounding effect of BMI on P-V curves include incorporation of a correction factor such as BMI or sagittal abdominal diameter when analyzing compliance curve parameters (28) or simultaneously measuring intra-rectal pressure as a surrogate index of intra-abdominal pressure (27).

Colonic compliance was dependent on the rate of inflation, implying that colonic compliance cannot be expressed by a single value. This observation, taken together with the exponential configuration of the quasi-static curve, and hysteresis suggest that the colon, like other biological materials is a viscoelastic body (20). Indeed, the observed exponential configuration of colonic P-V relationships in our study resembles the length-stiffness relationship in a computer-simulated linear viscoelastic model of smooth muscle (22).

Atropine attenuated differences between quasi-static and dynamic distensions, consistent with the hypothesis that rapid distension activated neural reflexes that reduced colonic compliance. These observations are germane to visceral perception because a preliminary report (8) suggests that the perception of rectal balloon distension is related not to distension per se, but to contractions induced by distension; rapid rectal distension, as opposed to slower distension, was more likely to induce contractions and to be perceived in healthy subjects and patients with irritable bowel syndrome. Thus the exaggerated perception of rectal balloon distension or “visceral hypersensitivity” in irritable bowel syndrome (34) may perhaps be secondary to differences in reflex contractile responses induced by rectal distension between health and irritable bowel syndrome. Whether the contractile responses are evoked by neural reflexes or by direct activation of smooth muscle is unknown.

In this study, we have characterized quasi-static colonic P-V relationships in healthy subjects. Further study is needed to compare quasi-static P-V curves...
during health and disease, e.g., slow-transit constipation, and, to obtain novel insights into the effect of inflation rate on viscoelastic properties by sinusoidal oscillations over a range of frequencies (12).

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