Adrenergic modulation of human colonic motor and sensory function

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Bharucha, Adil E., Michael Camilleri, Alan R. Zinsmeister, and Russell B. Hanson. Adrenergic modulation of human colonic motor and sensory function. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G997–G1006, 1997.—The effects of pharmacological modulation of adrenergic receptors on colonic motor and sensory function are unclear. We studied 40 healthy volunteers in a single-blind design; 12 received saline, and the remaining 28 received either clonidine, yohimbine, phenylephrine, or ritodrine. A barostat-manometric assembly in the left colon recorded drug effects on fasting and postprandial motor function, compliance, and sensation in response to standardized phasic balloon distensions delivered in random order. Clonidine reduced and yohimbine increased fasting, but not postprandial tone, by 63.2 ± 22.3% and 24.8 ± 8.8% (SE), respectively. Clonidine tended to reduce fasting phasic activity in the descending and sigmoid colon. A power exponential model provided the best fit to the compliance curve. Clonidine significantly increased colonic compliance. Clonidine reduced and yohimbine increased colonic perception of pain but not gas sensation during distension. Phenylephrine and ritodrine did not influence colonic motor or sensory function in the present studies. Thus α2-receptors modulate fasting colonic tone and compliance and alter perception of pain but not gas during mechanical stimulation of the colon.

colic tone; sensation; compliance

SYMPATHETIC STIMULATION is one of the putative mechanisms of paralytic ileus and even isolated colonic pseudo-obstruction (13). Conversely, pharmacological sympathetic blockade increases colonic motility in animals (14), and some patients with diabetic autonomic neuropathy have diarrhea that is attributable to loss of the "sympathetic brake." Clonidine, an α2-agonist, can ameliorate diabetic diarrhea (10); the precise mechanisms for this beneficial clinical response are unclear. α2-Agonists probably slow gut transit by enhancing α2-mediated fluid and electrolyte absorption (30) and by inhibiting fasting and postprandial small intestinal contractile activity (35, 36). However, the effects of α2-receptors and other adrenergic receptors on gut neuromuscular function in vivo require further elucidation. For example, the effects of pharmacological modulation of α2- and other adrenergic receptor subtypes on colonic motor and sensory function in humans are unknown. In addition to the effects of α2-receptors, the α1- and β2-receptors may also contribute to the overall effects of the sympathetic brake, since the α1-agonist phenylephrine and the β2-agonist terbutaline also reduce colonic contractile frequency in primates (26).

We have previously demonstrated that a pressure-clamped polyethylene balloon distended with air in the descending colon can measure colonic compliance, fasting tone, and its response to meal ingestion (11); the same balloon can be used as a distension stimulus to assess sensation (12). The colonic contractile response after ingestion of a meal has been demonstrated in several species, including humans (5, 11, 31). It has been suggested that a reduction in the tonic inhibitory sympathetic input to the colon contributes to the colonic meal response in primates (6).

The role of the adrenergic nervous system in mediation or modulation of colonic sensation in humans is unclear. α2- Receptors are found along nociceptive pathways in the spinal cord, brain stem, and forebrain (37), which are important relay stations in the three-neuron chain delivering visceral sensation from the colon to conscious perception. Moreover, although donidine has been used for postoperative analgesia (4), its effects on colonic sensation have not been evaluated.

In this study, our aims were to compare the effects of saline (control), donidine (α2-agonist), yohimbine (α2-antagonist), phenylephrine (α1-agonist), and ritodrine (β2-agonist) on colonic motility, compliance, and sensation in healthy human volunteers. We have not yet studied the β2-system with an antagonist, since a selective β2-agonist is unavailable for human studies.

MATERIALS AND METHODS

Healthy Volunteers

Forty healthy volunteers, aged 18–45 yr (mean ± SE, 29 ± 1.1 yr; 22 male and 18 female) were recruited by public advertisement. None had previously undergone abdominal surgery (other than an appendectomy and/or cholecystectomy). None was taking medications with the exception of acetaminophen or oral contraceptives. A clinical interview and physical examination were performed to exclude significant cardiovascular, respiratory, neurological, psychiatric, or endocrine disease. As in previous studies (12), validated screening questionnaires (a Bowel Disease Questionnaire (34) and the Hospital Anxiety and Depression Inventory (38)) were used to exclude subjects with irritable bowel syndrome and to determine anxiety and depression scores. All participants signed informed consent to participate in the studies, which were approved by the Institutional Review Board at the Mayo Clinic.

Administration of Drugs

Because of human safety considerations, the subject, but not the investigator, was blinded to the nature of the medication. Of the 40 subjects, 12 were randomized to receive placebo and 7 each to one of the other four agents. Subjects were informed they would be randomized to either a medication or placebo, either intravenous or oral. As control or...
placebo, we administered 10 ml of 0.9% saline as a “bolus” over 5 min, followed by an infusion at 40 ml/h for the entire study. The bolus volume and rate of administration were similar for yohimbine, the only other agent administered as a bolus followed by an infusion. Clonidine. Clonidine (Zenith, Northvale, NJ), 0.3 mg, was given as a single oral dose after at least 20 min of assessment of fasting colonic tone. This dose has previously been demonstrated to slow small intestinal and colonic transit and to enhance fluid absorption in healthy volunteers for at least 4.5 h (30). Intravenous clonidine is not available for administration to humans in the United States; however, the bioavailability of oral clonidine is nearly 100%. Peak concentrations and maximal antihypertensive effects are achieved at 1–3 h after oral administration (20); this peak time of action coincided with the planned experimental protocol in our studies. Cardiovascular recordings in our studies confirmed our expectation, since there was an obvious effect on colonic motor function after the systolic blood pressure had declined by >10 mmHg. This typically occurred 40–55 min after 0.3 mg clonidine had been administered orally.

Yohimbine hydrochloride. An intravenous formulation of yohimbine is not available in the United States. We obtained an IND (Investigational New Drug no. 46,250) from the Food and Drug Administration to use an aqueous solution of yohimbine, prepared by the Mayo Pharmacy from yohimbine hydrochloride (Sigma Chemical, St. Louis, MO). Stability of the aqueous solution of yohimbine over the 6-h period required to complete the study was checked by periodic high-performance liquid chromatography analysis. Yohimbine was administered as an initial 0.125-mg/kg intravenous bolus over 5 min followed by an infusion at 0.06 mg·kg\(^{-1}\)·h\(^{-1}\). Yohimbine is used clinically to provoke panic attacks in patients with a history of panic disorder (15); a similar dose stimulates central sympathetically outflowing plasma norepinephrine levels two- to threefold (15). The effects of yohimbine on fasting colonic tone were assessed during the intravenous bolus and during the subsequent infusion.

Phenylephrine. Phenylephrine (Elkins-Sinn, Philadelphia, PA), a selective \(\alpha_1\)-agonist, causes peripheral vasoconstriction, an increase in blood pressure, and reflex bradycardia. An initial dose of 0.4 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\) given intravenously was titrated upward in 0.4 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\) increments at 10-min intervals until the systolic blood pressure increased by 20 mmHg, or until a maximum infusion rate of 2.5 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\) was achieved. In four of seven volunteers randomized to phenylephrine, the dose at which the blood pressure end point was reached was maintained for the duration of the study. In the remaining three volunteers, the dose was titrated down during the study to maintain a heart rate of at least 45 beats/min, in accordance with limits set by our Institutional Review Board. For a 70-kg person, the median maximum dose of 84 \(\mu\)g/min (range 56–140 \(\mu\)g/min) was in the range recommended (40–180 \(\mu\)g/min) for treating hypotension in conditions such as hypovolemic or septic shock (21).

Ritodrine. Ritodrine (Abbott, Abbott Park, IL), a selective \(\beta_2\)-agonist, was infused intravenously at an initial dose of 50 \(\mu\)g/min, increasing by 50 \(\mu\)g/min at 10-min intervals until the heart rate had increased by 50% or a maximum dose of 350 \(\mu\)g/min was reached. In four of seven volunteers randomized to ritodrine, the dose at which the target heart rate was reached was maintained for the duration of the study; in the remaining three subjects, the dose was titrated so that the heart rate did not exceed 120 beats/min, as required by our Institutional Review Board. The dose range 100–300 \(\mu\)g/min and the dosing regimen are similar to those recommended for inhibiting uterine contractions in preterm labor, that is, 150–350 \(\mu\)g/min (22).

The effects of phenylephrine and ritodrine on fasting colonic motor function were measured for 20 min after the target systolic blood pressure and heart rate, respectively, were achieved.

**Hemodynamic Monitoring**

We continuously monitored arterial oxygen saturation, blood pressure, and cardiac rhythm using a pulse oximeter (CO2SMO, Novametrix Medical Systems, Wallingford, CT), Finapres sphygmomanometer (Ohmeda, Madison, WI), and electrocardiogram (Tektronix, Beaverton, OR), respectively. The blood pressure measurements were also confirmed by standard manual sphygmomanometer at regular intervals during the study.

**Colonic Motor Function**

A multilumen polyethylene balloon barostat-manometric assembly incorporating several manometric point sensors was placed into the prepared colon as described in previous studies (11, 12). Tonic and phasic contractile activity of the colon were measured as in previous studies (3, 32) using an inflatable compliant 10-cm-long balloon with a maximum volume of 600 ml (Hefty Baggies, Mobil Chemical, Pittsford, NY) linked to an electronic barostat (Distender Series II, G & J Electronics, Toronto, Ontario, Canada) which has a rigid piston. The manometric portion comprised six water-perfused (0.4 ml/min) pneumatic sensors, three in descending (sensor numbers 1–3) and three in sigmoid colon (sensor numbers 4–6); manometric sensors were 5 cm apart. The first and second sensors were 5 cm oral and caudal to the balloon, respectively. The balloon was positioned in the upper descending colon with the aid of flexible sigmoidoscopy and fluoroscopy. The intraballoon pressure at which respiratory excursions were regularly recorded as changes in barostat volume was defined as the “minimum distending pressure.” The “operating pressure” was set 2 mmHg above the minimum distending pressure (median pressure 10 mmHg, range 6–14 mmHg). Intraballoon volumes and manometric pressure changes in response to wall contractions and relaxations were monitored continuously throughout the study. A pneumobelt was applied around the abdominal wall at the level of the lower costal margin to help exclude artifact during movement and coughing.

**Colonic Compliance**

Colonic compliance was assessed as the volume response to 4-mmHg increments in intraballoons at pressures of 60-s intervals from 0 to 36 mmHg. The intrinsic compliance of the rigid piston in the barostat used for this study is nearly zero. Two compliance curves were performed, since intrasubjects visceral compliance may differ after an initial distension sequence and is more consistent after an initial distension, as previously shown in the stomach (2) and rectum (17). During the first compliance curve, intraballoon pressure was increased in 4-mmHg increments at 60-s intervals from 0 to 36 mmHg. After a 15-min equilibration period, a second colonic compliance curve was performed.

**Colonic Sensation**

As described previously (12), colonic distension was performed as rapid, intermittent “phasic” increases in intraballoon pressure in four steps of 8, 16, 24, and 32 mmHg greater than the operating pressure. The order of distensions
was randomized: each phasic pressure increment was maintained for 1 min, with a 1-min interval when the intraballoon pressure was reduced to operating pressure. For rating sensory perception, the participants were asked to mark two separate 100-mm visual analog scales for abdominal pain and feeling of gas at a standardized time, 20 s after the distension had commenced. The 100-mm-long visual analog scales were anchored at the ends by the descriptions “unnoticeable” and “unbearable.” During assessment of sensation, verbal interaction between the subject and investigator was kept to an absolute minimum. This approach was previously shown to be responsive to variations in stimulus intensity and to psychosensory modulation of symptoms (12).

The balloon volume during the initial part of each 1-min distension varies over time as the colonic segment accommodates initially to the pressure load. Therefore, the intraballoon volume was averaged over a 30-s period, beginning at 20 s after the distension had commenced. Levels of arousal and stress were determined immediately before assessment of colonic sensation, i.e., after drug administration. This was performed because a previous study in healthy participants (12) showed that the level of arousal significantly influences the perception of colonic distension. Thus two 100-mm linear analog scales marked “tired-energetic” and “active-drowsy” were used to assess levels of arousal, whereas levels of stress were assessed using similar scales marked “peaceful-tense” and “worried-relaxed” (12).

Colonic Response to a Meal

As in previous studies (11), colonic tone and motility were assessed for 20 min before and 90 min after consuming a chocolate milkshake containing 1,000 kcal (35% carbohydrate, 53% fat, and 12% protein).

Fig. 1. Experimental protocol.

Experimental Design

All subjects were admitted to the General Clinical Research Center at St. Mary's Hospital on the evening before the study for bowel preparation, comprising 1.5–2 liters of polyethylene glycol 3350 and electrolyte solution (OCL; Abbott Laboratories, Chicago, IL). This was drunk until the fecal effluent became a clear liquid. All volunteers signed informed consent forms and had a screening electrocardiogram to exclude significant rhythm disturbances or ischemia.

Women of child-bearing potential underwent a plasma β-human chorionic gonadotropin pregnancy test within 48 h of the studies. After an overnight fast, left-sided colonoscopy was performed without sedation. In the absence of any endoscopic abnormality, a 4-m Teflon-coated guidewire (Microvasive, Hobbs Medical, Stafford Springs, CT) was placed with its tip at the splenic flexure, and the colon was deflated as the colonoscope was withdrawn. The balloon-manometric assembly was introduced into the colon over the guide wire and positioned under fluoroscopic control with the barostat balloon in the mid-descending colon. After a 30-min equilibration period, the barostat operating pressure was set and the experiment started. The entire experimental protocol is summarized in Fig. 1.

Thirty minutes after setting the operating pressure, fasting colonic tone was measured for 40 min: 20 min before and 20 min after drug administration. This recording of fasting colonic tone was followed by measurement of colonic compliance, sensation, and the response to a standard meal.

Data Analysis

Colonic compliance. In analyzing the compliance curve, we averaged the barostat balloon volume over a 30-s interval. Therefore, there are two data points for each pressure increment lasting 60 s. The volume-pressure relationship obtained during a pressure ramp is often nonlinear. Therefore, a linear model may not detect differences between different segments of the compliance curve. Other investigators have used several mathematical functions, including a cubic polynomial model to “fit” the nonlinear shape (25). We examined several alternative nonlinear models and found the best fit was provided by a power exponential model, in which the volume (Vol) at any given pressure (P) is defined as

$$Vol = V_{max} \times \exp\left(-\frac{\beta}{P - 1/P_{max}}\right)$$

where relative pressure (RelP) = (1/P - 1/P_{max}), V_{max} is the maximum volume, and P_{max} is the maximum pressure in the compliance assessment. The parameter β reflects the overall

Fig. 2. Analysis of colonic compliance curves. Left: compliance curve for 1 individual. Note that because barostat volume was averaged over 30-s intervals, there are 2 data points at each “step.” Linear model provides a relatively poor fit to actual data points. Right: power exponential transformation of same data points on left with volume expressed as a proportion of maximum volume on y-axis and reciprocal of pressure on x-axis. This transformation and exponential analysis provides a better fit for compliance curve.
shape of the curve, and \( k \) is essentially the change in volume as a function of \( 1/P \) at any given point (Figs. 2 and 3).

The parameters \( k \) and \( b \) for the ascending portions (0–36 mmHg) of both colonic compliance curves were then estimated using the NLIN procedure in the SAS software package (29). Only the data from the second compliance curve were used for comparison between drugs. The estimated \( k \) and \( b \) for each subject were used to calculate the pressure corresponding to 10% of maximum volume (\( P_{10} \)) and half-maximum volume (\( P_{\text{half}} \)).

Colonic motor parameters. Phasic manometric pressure activity and changes in both pressure and volume of the barostat balloon were sampled as analog signals at 8 Hz and converted to digital signals before entry into a computer. As in previous studies, movement and respiratory artifact were filtered out using a modified VAX LAB filtering program (Ref. 11; Digital Equipment, Boston, MA). Thereafter, all data were analyzed by a customized computer program (11) that assessed phasic contractions and changes in the volume within the barostatically controlled balloon. On-line continuous recordings of all other parameters were similarly collected independent of the investigator.

For the manometric record, the waveform was initially smoothed using a high-frequency filter to remove instrumentation artifact. Thereafter, all contractions with a pressure change >10 mmHg amplitude above baseline, duration from 1.5 to 60 s, and an interpeak interval of >1.5 s were selected. These parameters are based on previous data showing that >98% of all colonic myoelectric activity has a frequency range of 1–11 min\(^{-1}\) (28). Computerized artifact removal was performed by excluding simultaneous peaks (to the nearest ±0.3 s), <5 s duration, and identification in at least five of the manometric channels. Phasic pressure activity was expressed as the motility index per hour as in previous studies (12) for the descending colon (sensors 1–3) and sigmoid colon (sensors 4–6).

Previous studies showed that phasic volume peaks recorded by the barostat balloon occur at a frequency of <3 min\(^{-1}\) (32); the waveform was filtered to remove frequencies >6 cycles/min, the barostat balloon volume was then computer analyzed to separate baseline balloon volume, representing colonic tone, from phasic volume deflections >10 ml from baseline volume.

Hemodynamic parameters were summarized as heart rate and mean arterial pressure (i.e., diastolic + pulse pressure)/3.

Statistical Analysis

Colonic tone and phasic activity were analyzed by similar methods. An overall comparison among groups for the various summary data values [e.g. colonic tone (baseline barostat volume), average motility index] was performed using an analysis of covariance adjusting for the predrug fasting values. For example, an analysis of covariance with the predrug mean fasting volume as a covariate was used to analyze the colonic response to the meal, which was expressed as the change between an average 20-min premeal value and a 90-min average value after the meal. In addition, five specific pairwise comparisons, between placebo and each of the four drugs, and between clonidine and yohimbine, were examined. Because saline and yohimbine were the only agents administered as a bolus followed by an infusion, a separate pairwise comparison between placebo and yohimbine was examined for a bolus vs. infusion effect using predrug values as a covariate.

The analysis of sensation scores (pain and gas) over the multiple distending pressures [0 (i.e., operating pressure), 8, 16, 24, and 32 mmHg] was based on a mixed model analysis of variance with drug group and actual barostat volume as effect variables, and with gender, anxiety, depression, tension, and energy scores as covariates. An unstructured variance-
covariance matrix was used to account for correlations among responses within the same subject at the multiple distending pressures.

RESULTS

Patient Characteristics

Demographic features, anxiety and depression ratings, as well as stress and arousal scores are summarized in Table 1. Scores for energy and tension, which were measured after drug administration, tended to be lower in volunteers who received clonidine. Because of an error in the computerized data transfer process, we lost all data from the first patient randomized to clonidine. Technical problems precluded an assessment of predrug fasting colonic volume in one volunteer.

Drug Effects on Hemodynamic Parameters

As indicated in Table 2, all drugs produced noticeable and statistically significant effects on mean arterial blood pressure and/or heart rate. In six volunteers who received either phenylephrine or ritodrine, the infusion rate was reduced to maintain the heart rate within the predesignated safety limits, i.e., at a heart rate >45 min⁻¹ with phenylephrine, or <120 min⁻¹ with ritodrine. No volunteer sustained adverse effects during the study.

Effect of Drug on Fasting Colonic Tone and Phasic Activity

An overall drug effect on fasting colonic tone was observed (P = 0.02). Clonidine reduced fasting colonic tone (P = 0.016) compared with saline (Figs. 4 and 5). Clonidine and yohimbine had significantly different (P = 0.001) effects on colonic tone. Similar drug effects on fasting phasic motor activity were observed in the descending (Fig. 6A) and sigmoid (Fig. 6B) colon. In particular, clonidine decreased while yohimbine increased fasting phasic contractile activity in the descending and sigmoid colon.

Because the overall comparison evaluated mainly the effects of a 20-min yohimbine infusion, we performed a separate pairwise comparison of bolus effects. The yohimbine bolus significantly reduced balloon volume, from 80.4 ± 12 to 68.2 ± 14.3 (SE) ml (P = 0.05), reflecting an increase in colonic tone when compared with saline bolus (predrug, 75.3 ± 8.4 ml; bolus, 72.1 ± 9 ml, P = NS). Moreover, we observed interindividual differences in the pattern of the tone response to yohimbine. Thus, in one volunteer, colonic tone increased dramatically during the bolus, with a recovery toward baseline during the infusion (Fig. 4). In four volunteers, the increase in tone during the bolus infusion was sustained or increased further during the infusion, whereas yohimbine did not affect colonic tone in one volunteer.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Feature</th>
<th>Placebo</th>
<th>Clonidine</th>
<th>Yohimbine</th>
<th>Phenylephrine</th>
<th>Ritodrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>31.1 ± 2</td>
<td>26.9 ± 1.7</td>
<td>25.9 ± 2.8</td>
<td>35.3 ± 2.3</td>
<td>26.7 ± 2.1</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>9:1</td>
<td>1:6</td>
<td>3:3</td>
<td>3:3</td>
<td>3:4</td>
</tr>
<tr>
<td>Energy, mm</td>
<td>23.9 ± 5.3 (10)</td>
<td>12.3 ± 8.2 (6)</td>
<td>43.3 ± 10.3 (6)</td>
<td>34.2 ± 4.9 (6)</td>
<td>29.3 ± 9.4 (7)</td>
</tr>
<tr>
<td>Tension, mm</td>
<td>23.6 ± 8.4 (10)</td>
<td>5.0 ± 2.2 (6)</td>
<td>17.0 ± 4.3 (6)</td>
<td>30.0 ± 12.5 (6)</td>
<td>43.0 ± 10.6 (7)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>2.1 ± 0.5 (10)</td>
<td>4.5 ± 1.6 (6)</td>
<td>5.3 ± 1.3 (7)</td>
<td>4.3 ± 0.7 (7)</td>
<td>3.6 ± 0.9 (7)</td>
</tr>
<tr>
<td>Depression</td>
<td>2.0 ± 0.5 (10)</td>
<td>3.3 ± 1.3 (6)</td>
<td>1.7 ± 0.6 (7)</td>
<td>1.7 ± 0.6 (7)</td>
<td>1.0 ± 0.5 (7)</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of patients given in parentheses. Anxiety and depression ratings were based on Hospital Anxiety and Depression Scale, where scores <7, 8–10, and ≥11 represent normality, indeterminate, and definite anxiety or depression, respectively.

Table 2. Effect of drugs on mean arterial pressure and heart rate

<table>
<thead>
<tr>
<th>Drug</th>
<th>MAP, mmHg</th>
<th>HR, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predrug</td>
<td>Postdrug</td>
</tr>
<tr>
<td>Placebo</td>
<td>129 ± 3</td>
<td>124 ± 3</td>
</tr>
<tr>
<td>Clonidine</td>
<td>128 ± 3</td>
<td>111 ± 3.6</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>127 ± 4</td>
<td>144 ± 5*</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>125 ± 6</td>
<td>166 ± 6*</td>
</tr>
<tr>
<td>Ritodrine</td>
<td>121 ± 3</td>
<td>164 ± 7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate. *P < 0.05 vs. predrug.

Fig. 5. Effect of drugs on fasting colonic tone. Histograms depict baseline barostat volume averaged over 20 min before (open bars; predrug) and after (solid bars; postdrug) drug administration. For saline (Pla) and yohimbine (Yoh), data reflect effects of infusion, and not bolus. Clo, clonidine; Phe, phenylephrine; Rit, ritodrine. •P = 0.016, clonidine vs. placebo.
Fasting colonic tone and phasic activity were not altered by phenylephrine or ritodrine.

Effect of Adrenergic Agents on Colonic Compliance

For the compliance curves, the goodness of fit using a power exponential model (median $r^2 = 99.2\%$), defined by the parameters $\kappa$ and $\beta$, was significantly better than for a simple linear model (median $r^2 = 96.4\%$; e.g., Fig. 2, overall comparison of $r^2$ values, $P < 0.001$). There were small, but statistically significant differences in the parameters $\kappa$ and $\beta$ derived from the ascending limbs of the first and second compliance curves (Table 3). The overall shape of the curve (Fig. 3A) is similar for all agents and is represented by the parameter $\beta$ (Table 4). The parameters $P_{10}$, $P_{\text{half}}$, and $V_{\text{max}}$, i.e., barostat pressures corresponding to 10 and 50% of maximum volume and the maximum volume achieved during the compliance curve, represent the initial, middle, and latter segments of the compliance curve, respectively. When compared with placebo, clonidine significantly reduces $P_{\text{half}}$ and $\kappa$ (representing the instantaneous slope of the curve; $P = 0.01$), but does not alter $\beta$ or $V_{\text{max}}$ (Table 4). For the $P_{10}$, although the overall test among groups (drugs) was not significant ($P = 0.10$), a pairwise comparison at an adjusted $\beta$-level of 0.0125 (for 4 tests) indicated clonidine was different from placebo ($P = 0.02$).

Effect of a Meal on Colonic Tone and Phasic Responses With Adrenergic Agents

The mean postprandial reduction in barostat balloon volume (consistent with increased colonic tone) for placebo was $40.8\pm 7.9\%$ (SE). After adjusting for predrug values of balloon volume, these drugs did not significantly alter the tonic or phasic response to a meal when compared with placebo (Table 5). Phenylephrine and clonidine enhanced the magnitude of the phasic response.

### Table 3. Comparison of first and second compliance curves for all subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First Curve</th>
<th>Second Curve</th>
</tr>
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<tbody>
<tr>
<td>$\kappa$</td>
<td>15 (1.7–44)</td>
<td>21.3* (7.3–40.3)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.9 (0.5–1.6)</td>
<td>1.0* (0.6–1.9)</td>
</tr>
<tr>
<td>$P_{\text{half}}$, mmHg</td>
<td>13.9 (3.3–22.3)</td>
<td>16.3* (8.2–23.5)</td>
</tr>
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</table>

Values are medians, with range values given in parentheses. $P_{\text{half}}$, pressure at half-maximum volume. *$P < 0.01$, signed rank test.
contractile to the meal response in the descending and sigmoid colon, respectively.

Effect of Adrenergic Agents on Perception of Colonic Distension

For placebo, the median pain and gas scores were proportional to pressure applied during balloon distension (Fig. 7, A and B). Of the potential covariates assessed (that is, gender, and median sensation scores (VAS) for anxiety, depression, stress, and arousal), only the anxiety score was a significant covariate in the analysis of abdominal pain sensation. None of these covariates was significant for the feeling of gas. Clonidine significantly reduced, whereas yohimbine increased VAS scores for abdominal pain, but no significant drug effects on the feeling of gas during balloon distension were detected.

DISCUSSION

This is the first study to comprehensively assess adrenergic modulation of colonic motor and sensory function and compliance in healthy human volunteers. Our data demonstrate that the \( \alpha_2 \)-agonist clonidine reduces fasting colonic tone and phasic activity, increases colonic compliance, and markedly attenuates the perception of pain, but not gas during colonic balloon distension. The marked hemodynamic and colonic effects of clonidine indicate that, as predicted from the high bioavailability and pharmacokinetic properties, the oral formulation was adequate for the purposes of this study.

The \( \alpha_2 \)-antagonist yohimbine increased fasting colonic tone and enhanced colonic perception of pain, but not gas. These drugs did not modify the overall colonic motor (tonic and phasic) response to a meal. Finally, the \( \alpha_2 \)-agonist phenylephrine and the \( \beta_2 \)-agonist ritodrine at the doses used in this study (which were maximal permissible doses in humans) did not affect colonic motor function, compliance, or sensation.

The opposing effects of clonidine and yohimbine on fasting colonic tone and sensation suggest that these effects are mediated via adrenergic \( \alpha_2 \)-receptors. It is conceivable that either central and/or peripheral imidazoline receptors mediate some of the effects of clonidine, which has an imidazoline ring structure (19). It is unlikely that the effects of yohimbine are mediated via imidazoline receptors, since the imidazoline-to-\( \alpha_2 \)-affinity ratio for this compound is \(<1:100\) (33). Similarly, studies in mice indicate that the agonist properties of clonidine at adrenergic \( \alpha_1 \) and \( H_2 \) histaminergic receptors are minimal, and they do not participate in the antidiarrheal effects of clonidine (7). Yohimbine is predominantly an \( \alpha_2 \)-antagonist; the non-\( \alpha_2 \)-effects of yohimbine at adrenergic \( \alpha_1 \), dopamine, and serotonin receptors probably do not contribute significantly to its effects at the dose employed in this study (15). Because both clonidine and yohimbine cross the blood-brain barrier, we cannot distinguish between central or peripherally mediated effects; previous animal studies using the peripherally active \( \alpha_2 \)-agonist ST-91, which does not cross the blood-brain barrier (36), or intracerebroventricular clonidine (7) suggest that the gastroin-

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### Table 4. Effect of drugs on colonic compliance

<table>
<thead>
<tr>
<th>Drug</th>
<th>( \kappa )</th>
<th>( \beta )</th>
<th>( P_{10}, \text{mmHg} )</th>
<th>( P_{\text{half}}, \text{mmHg} )</th>
<th>( V_{\text{max}}, \text{ml} )</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>23.8±2.6</td>
<td>1.0±0.1</td>
<td>7.8±0.9</td>
<td>17.2±0.9</td>
<td>234.8±11.7</td>
</tr>
<tr>
<td>Clonidine</td>
<td>12.5±2.1*</td>
<td>1.2±0.2</td>
<td>5.0±0.9‡</td>
<td>15.1±0.1‡</td>
<td>270.2±41.4</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>18.4±2.2</td>
<td>1.1±0.1</td>
<td>6.7±0.8</td>
<td>14.6±1.1</td>
<td>227.2±14.9</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>26.5±3.2</td>
<td>1.0±0.1</td>
<td>8.3±0.7</td>
<td>18.4±1.2</td>
<td>219.2±13.5</td>
</tr>
<tr>
<td>Ritodrine</td>
<td>21.1±3.3</td>
<td>1.0±0.1</td>
<td>7.0±0.7</td>
<td>16.1±1.6</td>
<td>219.4±19.7</td>
</tr>
<tr>
<td>Overall test</td>
<td>( P &lt; 0.05 )</td>
<td>NS</td>
<td>( P = 0.1 )</td>
<td>( P &lt; 0.05 )</td>
<td>NS</td>
</tr>
<tr>
<td>Pairwise comparisons vs. saline§</td>
<td>( P = 0.005^* )</td>
<td>( P = 0.02^* )</td>
<td>( P = 0.002^* )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. §Adjusted \( \alpha \)-level is 0.0125 (for 4 tests). \( P_{10} \) and \( P_{\text{half}} \), pressures at which barostat balloon volume was 10% and half-maximum balloon volume, respectively; \( V_{\text{max}} \), maximum balloon volume reached during colonic compliance curve. Note that because volume is plotted against 1/pressure, a lower \( \kappa \) value represents increased compliance with clonidine.

### Table 5. Effect of drugs on tonic and phasic colonic meal response

<table>
<thead>
<tr>
<th>Drug</th>
<th>Average Barostat Volume, ml</th>
<th>Average Phasic Activity, MI/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premeal</td>
<td>Postmeal</td>
</tr>
<tr>
<td>Placebo (10)</td>
<td>78.8±9.7</td>
<td>41.9±7.5</td>
</tr>
<tr>
<td>Clonidine (6)</td>
<td>176.7±23.2</td>
<td>144.4±30</td>
</tr>
<tr>
<td>Yohimbine (6)</td>
<td>105.7±13</td>
<td>63.8±1.1</td>
</tr>
<tr>
<td>Phenylephrine (6)</td>
<td>75.7±16.2</td>
<td>53.2±19.4</td>
</tr>
<tr>
<td>Ritodrine (6)</td>
<td>104.3±20.2</td>
<td>75.8±18.9</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of experiments given in parentheses. MI, motility index. Premeal barostat volume is a 20-min average immediately before meal. Absolute values are different from fasting volumes in Fig. 5, which were obtained before assessment of compliance and sensation. Overall comparison among groups of post- vs. premeal: for barostat volume, \( P = 0.22 \). *\( P = 0.02 \), †\( P = 0.05 \), for difference (post – pre) vs. placebo.
testinal motor effects of $\alpha_2$-agonists are partly peripherally mediated.

The opposing effects of clonidine and yohimbine on fasting colonic tone are consistent with the recognized ability of $\alpha_2$-adrenergic receptor agonists to inhibit acetylcholine release from neurons in the myenteric plexus and from the parasympathetic pelvic plexus, thereby inhibiting gastrointestinal motility (14). Thus $\alpha_2$-antagonists enhance baseline colonic motility in cats (14). In contrast, the $\alpha_2$-agonists (either clonidine or ST-91) inhibit defection in mice (7), reduce baseline colonic contractile activity in primates (26), and inhibit small intestinal and colonic transit in human volunteers (30).

Several reasons may account for the interindividual variability in the effects of yohimbine on fasting colonic tone. Plasma levels of yohimbine and, consequently, norepinephrine are higher during a yohimbine bolus than during a subsequent infusion (18). Interindividual variations in baseline sympathetic tone may influence the response to yohimbine. Indeed, the hemodynamic variations in baseline sympathetic tone may influence the response to yohimbine. Hemodynamic variations in baseline sympathetic tone are greater in humans with a history of anxiety, depression, or other psychopathology (16). Finally, Gillis et al. (14) observed that the increase in colonic motility after nonspecific sympathetic blockade lasted for only 6 min, indicating the possibility of tachyphylaxis. It is conceivable that acetylcholine released from myenteric cholinergic neurons in response to an $\alpha_2$-antagonist feeds back to inhibit neuronal acetylcholine release, which in turn limits the duration of enhanced contractility (14).

Clonidine did not attenuate the tonic or phasic colonic meal response, suggesting that a physiological stimulus can overwhelm the inhibitory effects of clonidine on fasting motor activity. Furthermore, it seems unlikely that a reduction in $\alpha_2$-mediated sympathetic input, as suggested by Dapoigny et al. (6), is the sole or major mechanism for the colonic meal response. It is also conceivable that the failure of clonidine to influence the meal response may have resulted from a decline in plasma clonidine levels over the 2-h period that elapsed between measurement of fasting and postprandial colonic tone. However, we consider this unlikely, since systolic blood pressure remained significantly lower than baseline throughout the postprandial period.

Our studies provide interesting insights on colonic compliance in humans. With the use of an infinitely compliant polyethylene balloon and a rigid barostat cylinder, the colonic pressure-volume curve is approximated by an exponential function. The barostat used in this study obviated the need to correct for the intrinsic compliance of a latex balloon which is nonlinear, or for the compliance of the rubber “bellows-type” barostat. The vast majority of papers have previously summarized gastric, colonic, and rectal pressure-volume relationships using either a linear or cubic polynomial function (11, 25). An exponential function provided the best fit to the actual data points in our study and, in contrast to the linear functions, can distinguish between the effects of placebo and clonidine on colonic compliance. An exponential function provides an opportunity to differentiate the initial component from the latter portions of the compliance curve. Also, in contrast to a linear function, the exponential function indicates that, within defined limits of pressure loads that apply in the in vivo setting, colonic volume reaches a near maximum and does not increase despite increases in balloon pressure.

Mertz et al. (23) suggested that “active” compliance, representing muscular tone, and relaxation primarily contribute to the pressure-volume relationship during the initial portion of the curve. In contrast, the latter part of the curve at higher balloon pressures reflects the viscoelastic properties of connective tissue and muscle, i.e., “passive” compliance, which provides the primary resistance to stretch. According to this hypothesis, pharmacological agents would be anticipated to alter the initial but not the latter portion of the compliance curve. The latter portion may be more clearly influenced by fibrosis or inflammation, which change the colon’s viscoelasticity. The exponential model indicates that clonidine does not alter the overall shape of the curve (or $\beta$), but does alter $\kappa$, or instantaneous slope of volume expressed against 1/pressure. A close perusal of colonic compliance curves, as depicted in Fig. 3A, demonstrates an initial flat portion, a transition to a steeper segment at an “inflection point,” and a flatter segment at the highest pressures tested. Therefore, we selected the parameters $P_{10}$, $P_{1/2}$, and $V_{\text{max}}$ to represent the initial, middle, and latter portions of the compliance curve, respectively. Although the $P_{10}$ indicates a leftward shift in the compliance curve (see Fig. 3B), it is important to note that the instantaneous slope of the curve ($\kappa$) is also significantly altered by clonidine. Thus clonidine changes compliance both at low pressures (“active compliance”) and along the whole range of pressures tested, possibly reflecting viscoelastic properties of the colon. In contrast, clonidine did not change the maximum balloon volume recorded during imposed pressures of 36 mmHg. This parameter, $V_{\text{max}}$, is similar for all agents, suggesting that drug-related differences in active compliance are unlikely to influence pressure-volume relationships at the highest pressures tested.

The opposing effects of clonidine and yohimbine on balloon distension-induced pain, but not gas sensation, were impressive. These effects may result from a combination of drug effects on colonic tone and/or nociceptive pathways. Clonidine reduced colonic tone and sensation, whereas yohimbine increased colonic tone and sensation. Similarly, colonic sensitivity also increases after a meal when tone is generally increased (9). It has been hypothesized that alterations in viscus tone alter sensitivity by altering the set point or threshold of receptors in the gut wall (24). However, if the sensory effects of clonidine and yohimbine were predominantly mediated by alterations in tone, one would anticipate similar effects on gas and pain sensation, since visceral receptors for noxious and nonnoxious sensation are probably situated at similar levels in the gut wall. Therefore, our data do not support a sensory effect mediated solely through changes in tone.
but suggest a specific effect on afferent pathways. However, this hypothesis deserves further study with in-depth dose-response studies of \( \alpha_2 \)-agents on colonic sensation, motility, and tone.

There are several a priori reasons to support an effect of \( \alpha_2 \)-agents on pain pathways independent of changes in tone. \( \alpha_2 \)-Receptors are situated at multiple locations along the noxious pathway in the spinal cord, brain stem, and forebrain (37); the antinociceptive properties of clonidine in humans with somatic pain are well recognized (4). Volunteers who received clonidine in our study tended to have lower VAS scores for arousal and stress, suggesting an effect on the central nervous system. However, clonidine did not induce sleep, and all subjects receiving clonidine were awake throughout the studies. Further experiments are required to determine whether central opioid receptors might be involved in mediating the antinociceptive effects of clonidine during colonic distension.

Neither phenylephrine nor ritodrine significantly altered colonic motor or sensory function using a dose range modified according to recorded hemodynamic parameters. Our findings are consistent with observations in primates where the higher doses of phenylephrine \( >50 \mu g/kg \) were required to reduce colonic contractile activity (26). Because of the risks of hemodynamically adverse consequences, we were unable to use a higher dose in healthy subjects. It is possible that higher doses that cannot be safely used in humans could alter colonic tone and/or sensation. Although postsynaptic \( \beta_2 \)-receptors are found in the cat colon (8), ritodrine did not modify colonic tone or sensation at the dose that is known to relax uterine smooth muscle in humans (22). Our experiments did not assess the effects of modulating \( \beta_1 \)-receptors that are found in the cat colon (8) and inhibit primate colonic contractile activity (26). The selective \( \beta_1 \)-antagonist metoprolol increases sigmoid colonic pressure activity, and the effects of selective \( \beta_3 \)-receptor modulation on colonic physiology deserve further study (1).

In summary, these observations on colonic motor and sensory effects of adrenergic agents suggest that \( \alpha_2 \)-agents should be studied further, including evaluation of their effects on transit and sensation. Eventually, they may be useful additions to our therapeutic armamentarium for patients with alterations in colonic motor and sensory function such as irritable bowel syndrome. \( \alpha_2 \)-Antagonists have been successfully employed to date for managing paralytic ileus [narcotic bowel syndrome (27)]. These and other potential indications, such as in the treatment of megacolon, require further study. Further studies are also needed to clarify the role of imidazoline receptors, the contributions of central vs. peripheral \( \alpha_2 \)-receptors, and the role of \( \alpha_1 \)-, \( \beta_1 \)-, and \( \beta_3 \)-receptors in modulating colonic motor and sensory function.

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