Effects of an \( \alpha_2 \)-adrenergic agonist on gastrointestinal transit, colonic motility, and sensation in humans

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A NUMBER OF GASTROINTESTINAL diseases, including functional bowel disorders, is characterized by disorders of colorectal motor and sensory function (8). Heightened sensitivity has been demonstrated in irritable bowel syndrome (IBS [25, 28]). Current therapy for these conditions is suboptimal; future advances require mechanistic studies of the neuromuscular apparatus of the colon. Previous studies suggest that the adrenergic nervous system provides extrinsic tonic inhibitory control of gut motility. Visceral afferents ascend along sympathetic nerves to enter the spinal cord at the dorsal horn; descending adrenergic and serotonergic fibers in the spinal cord modulate dorsal horn neuron function, thereby altering ascending transmission mediating visceral sensation (9).

Correction of sensory dysfunction or visceral nociception are current targets of therapy in functional gastrointestinal disorders. There is a need for selective therapies that modulate sensory functions without deleterious effects on other functions, such as the motor function or the affect. We have previously demonstrated that, among a variety of adrenergic agents active on receptor subtypes, the \( \alpha_2 \)-adrenergic agents, administered at maximal approved doses, affect human colonic (4, 23) and rectal (23, 24) motor and sensory function. Thus clonidine, an \( \alpha_2 \)-adrenergic agonist, induces colonic and rectal relaxation and reduces conscious perception of balloon distension in the colon and rectum (4, 23, 24). On the other hand, yohimbine, an \( \alpha_2 \)-adrenergic antagonist, has been shown to cause contraction of the rectum and to increase the perception of balloon distension in the rectum (23, 24).

Animal studies had previously demonstrated significant effects of clonidine on gastric emptying (18). Clonidine rarely causes pseudoobstruction (36), and, at a dose of 0.3 mg b.i.d., it has previously been shown to be effective in the treatment of diabetic diarrhea, at least partly through its effects on \( \alpha_2 \)-adrenergic receptors on enterocytes (12, 15). Our hypothesis was that clonidine would reduce colonic sensation in response to distension, but this would be achieved with doses that significantly retard gastric, small bowel, or colonic transit. In view of the location of \( \alpha_2 \)-adrenergic receptors on visceral afferents or spinal neurons, a second hypothesis was that the effects of clonidine on sensation would be demonstrable at the noxious end of the sensation range, consistent with the demonstration that visceral afferents function as wide dynamic range neurons, whereas vagal afferents encode stimuli in the physiological range (32, 33). Thus our aims were to further characterize dose-related effects of clonidine on colonic motor and sensory function and gastrointestinal and colonic transit.
MATERIALS AND METHODS

Healthy Volunteers

Fifty-five healthy volunteers, aged 20–57 yr (20 males, 35 females; mean age, 36 yr), were recruited by public advertisement. None had undergone previous gastrointestinal surgery; all had negative responses on the Bowel Disease Questionnaire (37) and normal responses on the Hospital Anxiety and Depression Inventory (39) as well as a normal clinical and physical examination and echocardiogram. Women of childbearing potential were required to have a negative pregnancy test. The protocol was approved by the Mayo Institutional Review Board, and written informed consent was given in all cases.

Experimental Design

We performed a parallel-group, dose-response study with 55 subjects participating in the gastrointestinal transit studies; these were randomized in single-blind fashion (as required by our ethics committee) to clonidine treatment as follows: placebo, n = 11; 0.025 mg, n = 11; 0.5 mg, n = 11; 1.0 mg, n = 9; 2.0 mg, n = 8; or 3.0 mg, n = 5. Nineteen of these participants also consented to colonic intubation studies. For participation in the colonic intubation and transit studies, the colonic intubation was performed on day 1, before any medication. Baseline (predrug) measurements of compliance, tone, and sensation were performed. The medication was then administered, and postdrug measurements were repeated 1 h later. A separate group of 16 participants underwent colonic intubation studies only; the results of this group have been previously evaluated and published (39). Combining the two groups that underwent intubation studies (n = 35), the treatment randomization was as follows: placebo, n = 11; 0.025 mg, n = 6 each; 0.05 mg, n = 7; and 0.3 mg, n = 4. Participants were dosed with medication daily at the same time in the morning ½ h before breakfast for 7 days; a 48-h transit test was performed on days 5–7. If the participant consented to colonic intubation studies, the colon was cleansed (see below) by oral colonic lavage solution after the final transit scan had been obtained. For those 36 participants who only underwent the gastrointestinal and colonic studies, these were scheduled for day 5 ± 2 after start of medication. Table 1 shows the number of subjects in each treatment arm and the doses of clonidine given.

Gastrointestinal Transit Studies

Procedure. On the day of transit measurements, the study medication was administered at a standardized time, 60 min before administration of the capsule containing radiolabeled particles. An adaptation of our established scintigraphic method was used to measure gastrointestinal and colonic transit (6, 7, 10, 11, 26). Briefly, 111In adsorbed on activated charcoal was delivered to the colon by means of a methacrylate-coated, delayed-release capsule (6). The capsule was ingested following an overnight fast. After the capsule emptied from the stomach (documented by its position relative to radioisotopic markers placed on the anterior iliac crests), a radiolabeled meal was ingested. In this meal, 99mTc-sulfur colloid was used to label two scrambled eggs, which were eaten with one slice of whole wheat bread and one glass of whole milk (300 kcal). This meal facilitated measurement of gastric and small bowel transit. Subjects ingested standardized meals for lunch and dinner at 4 and 8 h after the radiolabeled meal. We obtained abdominal images, relative to the time of meal ingestion, every 15 min for the first 2 h, then every 30 min for the next 4 h, and performed scans at 8, 24, and 48 h.

Transit data analysis. A variable region of interest program was used to quantitate the counts in the stomach and each of four colonic regions: ascending, transverse, descending, and combined sigmoid and rectum. These counts were corrected for isotope decay, tissue attenuation, and cross-talk (downscatter) of 111In counts in the 99mTc window (11, 26).

The geometric center is the weighted average of counts in the different colonic regions [ascending (AC), transverse (TC), descending (DC), rectosigmoid (RS)] and stool. At any time, the proportion of colonic counts in each colonic region is multiplied by its weighting factor as follows:

\[ \frac{\%AC \times 1 + \%TC \times 2 + \%DC \times 3 + \%RS \times 4 + \% stool \times 5}{100} \]

Thus a high geometric center implies faster colonic transit; a geometric center of 1 implies that all isotope is in the ascending colon, and a geometric center of 5 implies that all isotope is in the stool. We also estimated the t1/2 of emptying of the ascending region.

Colonic Intubation Studies

Procedure. All subjects were admitted to the General Clinical Research Center on the evening before the study for bowel cleansing with 2 liters of polyethylene glycol and electrolyte solution (OCL; Abbott Laboratories, Chicago, IL) and overnight fast. Left-sided colonoscopy was performed without sedation; a 4-mTeflon-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 colonic tube assembly was introduced into the colon over the guidewire and positioned under fluoroscopic control with the polyethylene balloon [9-cm-long cylinder with a maximum volume of 600 ml (Hefty Baggies; Mobil Chemical, Pittsford, NY)] in the middescending colon. Blood pressure was recorded at 30-min intervals during the entire study. Patients lay in the right lateral position during the study to avoid pressure on the descending colon from surrounding organs.

After a “dummy” distension (ramp to 20 mmHg with steps of 4 mmHg, 30 s), which has previously been shown to enhance reproducibility of colorectal compliance tests (19), the barostat operating pressures were set (2 mmHg above the point at which respiratory variation was noted). After a 30-min wait, the levels of sensory perception and compliance were measured, followed by fasting tone (30-min period). Clonidine was then administered, and postdrug tone (30 min), compliance, and perception were reassessed. Tone was assessed (35) for 30 min premeal and 1 h after a 1,000 kcal, liquid, high-fat (50%) meal.

Colonic motor function. Tone of the colon was measured as in previous studies (7) as the baseline balloon volume averaged for 30 min during fasting and for the first postprandial hour.

Colonic compliance. Colonic compliance was assessed as the volume response to 2-mmHg increments in intraballoon pressures at 30-s intervals from 0 to 24 mmHg above operating pressure. The rigid piston barostat used in this study (Distender Series II; G & J Electronics, Toronto, Ontario, Canada) has almost zero intrinsic compliance.

Colonic sensation. Subjects received a standardized information sheet before sensation testing; thereafter, there was
minimal interaction between subject and investigator. Sensation was assessed by responses recorded on a visual analog scale during rapid phasic distensions of 8, 16, and 24 mmHg above operating pressure performed in a randomized order. Each distension lasted 60 s and was followed by a rest period at the operating pressure, also lasting 60 s. Ratings of sensory perception were assessed at a standardized time, 30 s after the onset of the distension. The subject was asked to record perception on three 100-mm visual analog scales for the feeling of gas and pain during colonic distensions. The visual analog scales were anchored at each end by the descriptions “none” and “worst possible.” This approach to measuring visceral perception has previously been shown to be sensitive to decreases in pain scores during studies using identical methods [including the visual analog scale (VAS) scores] in healthy volunteers (4, 16). A second rationale for the separate assessments of sensation at different distension pressures is provided by the evidence from Sengupta et al. (32, 33) that vagal afferents convey visceral afferent signals in the physiological range, whereas visceral afferents operate over a wide dynamic range encoding noxious stimuli. A secondary hypothesis of the study addressed the question of whether clonidine was modulating colonic sensation in the physiological or noxious range of afferent stimulation.

**Statistical Analysis**

A summary (mean, median, standard error, minimum, and maximum) of the predrug values for colonic tone (volume), compliance, sensation scores (on the 10-cm VAS scale), and transit measures was compiled for each clonidine dose and placebo group. Transit measures were analyzed using the Kruskal-Wallis test to assess overall differences among dose groups. The overall effects of dose on compliance curve summary parameter values (κ, β, and Pr1/2) were assessed by ANOVA. Two specific contrasts were also tested: one for overall effects (all doses vs. placebo) and one for linear dose effects. Similarly, analysis of covariance was used to assess drug and dose effects on postdrug colonic tone values incorporating predrug tone values and body mass index measures as covariates. ANOVA was used to assess drug and dose effects on colonic tone in response to a meal, based on the difference in the natural log of colonic tone values (premeal tone minus loge postmeal tone). The corresponding mean values per group were summarized as “symmetric percent differences” [and their 95% confidence intervals (CI)] obtained from multiplying the difference in loge values by 100 (14).

**Fig. 1.** Effects of several doses of clonidine on gastrointestinal and colonic transit. Note that clonidine does not significantly alter gastric emptying at 2 h nor colonic filling at 6 h, a surrogate for small bowel transit when gastric emptying is not altered.
The VAS recorded sensation scores for gas, pain, and aggregate (i.e., average of gas and pain) were analyzed after first transforming the scores to rank scale overall doses and distension levels separately for the pre- and postdrug periods. This was done to compensate for the skewed distribution of sensation scores at several distension levels. Then the differences (postdrug minus predrug) were computed for each subject at each distension level. The differences were analyzed using an ANOVA at each distension level (8, 16, and 24 mmHg). In addition, simultaneous comparisons over all distension levels were also made using multivariate tests. Finally, the mean gas, pain, and aggregate scores before and after drug administration over all three distension levels were also computed for each subject. These mean scores were then transformed to the rank scale, and the differences (postdrug minus predrug) were analyzed by ANOVA without multivariate comparison.

The association between changes in colonic tone and compliance with the changes in colonic sensation (pre- vs. postclonidine treatment) was assessed by using (Spearman) rank correlation coefficients.

RESULTS

Participant Characteristics

Demographic features and hospital anxiety and depression ratings are summarized in Table 1; none of the scores was in the range associated with clinically significant affective disorder.

Effects of Clonidine on Gastrointestinal and Colonic Transit

No statistically significant effects of clonidine on transit through the stomach, small bowel, or colon were detected, as demonstrated in Figs. 1 and 2.

Effects of Clonidine on Colonic Compliance

The fit of the power exponential model across all studies resulted in a median $R^2 > 0.95$. No statistically significant predrug differences in compliance were detected among the different dosage levels of clonidine and placebo. Examples of compliance curves pre- and postplacebo and two doses of clonidine are shown in Fig. 3.

Clonidine induced an overall increase in colonic compliance ($P = 0.023$) as characterized by increased values of $\kappa$, and illustrated by the shift of the compliance curve to the left, resulting in lower $Pr_{1/2}$ postdrug, which reflects greater colonic compliance (Fig. 4). This also reflects an increase in fasting volumes (or reduced tone). The effects of clonidine on compliance parameter $\kappa$ showed a significant linear dose-related effect ($P = 0.004$).
Effects of Clonidine on Colonic Tone

There were no differences in the predrug balloon volumes (tone) among the different dosage levels of clonidine. A simple paired *t*-test (postdrug vs. predrug) indicated greater volumes postdrug [overall mean (95% CI) 10.7 ml (1.4, 20.1), *P* < 0.05]. Figure 5 shows fasting levels of postdrug colonic tone. The unadjusted (for covariates) mean changes in volumes for each dose are shown in Table 2; note that the 0.05-mg dose produced a mean change in volume (8.1 ml) that almost reached the overall mean (10.7 ml). A statistically significant linear dose-related effect on postdrug tone was not detected, but an overall drug effect on tone was observed (*P* < 0.05).

The meal induced a reduction in balloon volumes (increase in tone) in the colon (*P* < 0.05; overall dose groups, the mean percent symmetrical difference was 41.8 ± 6.2%); however, there was no significant overall drug (vs. placebo) or dose-related effect compared with placebo on the colon’s tone following the meal (Fig. 6).

Effects of Clonidine on Colonic Sensation

The aggregate (combined) scores of gas and pain are provided in Table 2. There were significant effects of clonidine on aggregate sensation scores and, individually, gas and pain scores (Fig. 7). In general, these effects were dependent on the distension pressures, as detailed below. The effects of clonidine (vs. placebo) at individual pressure distensions were univariately significant only at 24 mmHg distension (*P* = 0.021). However, the multivariate analysis and the analysis of mean scores over the three distension levels were significant (*P* = 0.046 and *P* = 0.013, respectively) for the overall drug vs. placebo comparison.

There were significant linear dose-response effects of clonidine on aggregate sensation score at 24 mmHg (*P* = 0.013) and for the mean scores over all three distension levels (*P* = 0.004). The multivariate analysis also indicated significant linear dose effects (*P* = 0.01).

Clonidine significantly reduced mean (over all 3 distension levels) gas sensation score (*P* = 0.028), and a significant linear dose-response was observed (*P* = 0.017). The overall effects of clonidine vs. placebo on pain scores at individual distension levels or the mean (over the 3 levels of distension) were not significant. However, we observed linear dose-response effects of clonidine at a distension of 24 mmHg (*P* = 0.04) and for the mean pain score over three distension levels (*P* = 0.053). There were reductions in pain scores with the 0.1- and 0.2-mg doses of clonidine (Fig. 7); however, the effects of 0.3 mg clonidine on pain sensation (Fig. 8) reflected the main antinociceptive activity of the medication.

No significant associations between changes in tone and compliance vs. changes in sensation scores (pre- vs. postclonidine treatment) were detected.

DISCUSSION

This study provides further evidence that the α2-adrenergic system alters motor and sensory function of the human colon in healthy individuals. As in our previous studies, we confirmed that clonidine significantly altered colonic fasting tone, compliance, and sensation of pain during mechanical distension. Moreover, the present study also advances our understanding of the potential therapeutic window for clonidine in

Table 2. Mean change in postdrug fasting barostat balloon volume

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose, mg</th>
<th>Predrug</th>
<th>Postdrug</th>
<th>Mean Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td>121.4</td>
<td>105.4</td>
<td>-15.9 (−25.8, −6.0)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.025</td>
<td>122.2</td>
<td>129.0</td>
<td>6.8 (−30.3, 43.8)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.05</td>
<td>90.4</td>
<td>98.5</td>
<td>8.1 (−22.4, 38.6)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.1</td>
<td>106.8</td>
<td>135.2</td>
<td>28.4 (0, 56.5)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.2</td>
<td>97.3</td>
<td>117.9</td>
<td>20.6 (0, 41.4)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.3</td>
<td>134.1</td>
<td>153.6</td>
<td>19.5 (7.7, 31.3)</td>
</tr>
</tbody>
</table>

CI, confidence interval.
the treatment of functional gastrointestinal disorders in view of the in depth dose-response studies performed. We have shown that doses that reduced aggregate sensation of gas and pain and colonic tone and increased compliance do not deleteriously affect gastrointestinal motility, such as transit and postprandial colonic tone. Three other completely novel observations in the present study are that, at doses permissible in humans, 1) clonidine does not significantly alter gastrointestinal or colonic transit, 2) clonidine reduces sensation of gas over the three distension pressures, and 3) there are significant linear dose responses in the effects of clonidine on colonic sensory and motor responses. It is, however, important to note that the dose responsiveness for sensation was evident over the entire dose range with lower levels of mechanical distension (8 mmHg) but was predominantly influenced by the highest dose of clonidine (0.3 mg) for the 24-mmHg distensions (see Table 3).

These studies indicate threshold doses as low as 0.1 mg for relaxing the colon and dose-related reduction of gas sensation in response to distensions. Significant effects on pain were only observed with 0.3 mg clonidine. If adrenoreceptor modulation with \(\alpha_2\)-agonists is to have any potential application to clinical therapeutics of functional gastrointestinal disorders, it is essential that there are no adverse effects on the gut. Hence the lack of any significant effects of clonidine (up to a 0.3-mg dose) on gastric, small bowel, or colonic transit and the ability of the standard meal to induce a normal increase in tone are encouraging.

Our current data confirm previous observations from our laboratory that clonidine has a significant effect on tone and compliance (4, 23), but they extend previous reports because we have shown significant dose-related effects on compliance, aggregate sensation, and gas scores during mechanical distension experiments. Our data suggest that the sensory or perceptual responses to mechanical distension observed during clonidine treatment may be partly dependent on the effects on compliance since both aggregate sensation and compliance show dose relationships to the effects of clonidine. Clonidine doses as low as 0.05–0.1 mg may be sufficient for relief of gas sensation, especially during low pressures of distension (e.g., 8 mmHg above the baseline operating pressure), and these doses of clonidine appear to be worthy of further study in patients with functional gastrointestinal disorders, particularly since these doses have less potential to cause hemodynamic side effects and they do not significantly alter gastrointestinal or colonic transit.

Clonidine lowered pain sensation in the colon at all levels of distension. Although there was a significant linear dose response, Fig. 7 illustrates that the reduction in pain score was observed with the 0.1- and 0.2-mg doses but was greatest with the 0.3-mg dose. In fact, the effect on pain was only significant at the
0.3-mg dose relative to placebo. These data expand on the previous observations (4) from our lab showing that 0.3 mg po clonidine reduced pain sensation during colonic distension. In our initial study, clonidine failed to significantly alter gas sensation (4); however, the larger study reported here shows that there is a significant effect on gas sensation during mechanical distension in the higher range of pressures. These observations confirm the importance of α2-adrenergic mechanisms in the control of colonic sensation and suggest a potential role for clonidine in the treatment of hypersensitivity or hypercontractile states in the colon, such as in patients with diarrhea-predominant IBS or colonic autonomic neuropathy. These conditions are associated with rectal hypersensitivity (25) and increased prevalence of high-amplitude propagated colonic contractions (13), respectively. Since the Spearman rank correlations between changes in tone or compliance vs. changes in sensation scores were not significant, it appears that the effects of clonidine on sensation are less likely to be associated with changes in motor responses of the colon. This observation is consistent with the hypothesis that clonidine’s sensory effects result from changes in visceral afferent function.

Sympathetic dysfunction is associated with diarrhea-predominant IBS (13) or slow-transit constipation (1, 3). Three human α2-adrenoceptor subtypes have been cloned and characterized and are denoted as the 2A, 2B, and 2C subtypes (21, 22, 27). Based on chromosomal localization, these have previously been denoted as 2C10, 2C2, and 2C4, respectively. Recent studies, including those with genetically engineered mice, have shown that the 2C subtype plays specific roles in modulation of the acoustic startle reflex, isolation-induced aggression, spatial working memory, development of behavioral despair, body temperature regulation, dopamine and serotonin metabolism, presynaptic control of neurotransmitter release from cardiac sympathetic nerves and central neurons, and postjunctional regulation of vascular tone (5, 17, 20, 29–31, 38). Del322–5, a polymorphism in the third intracellular loop of the α2C-adrenoceptor, results in a loss of several signal transduction cascades [mitogen-activated protein kinase 71% impaired and inositol phosphate 60% impaired (38)] and may contribute to pathophysiology. The therapeutic utility of α2-adrenoceptor agonists and antagonists has been limited by the lack of highly subtype-specific compounds as well as marked interindividual variability in efficacy and adverse side effects of available agents. The interindividual variability in responses may reflect pharmacogenic differences; in a preliminary study of 22 patients with diarrhea-predominant IBS, we have observed that the allele frequency of the Ncl1 polymorphism tested for in the α2C-adrenoceptor was 0.238 compared with the previously published frequency of 0.040 in a healthy Caucasian population (2). In contrast, the same study failed to demonstrate polymorphisms in α2A-adrenoceptor or mutations in the gene for norepinephrine transporter protein (2). Although the preliminary observations clearly require confirmation, α2-adrenoceptor genotypic variance in patients and the effects of clonidine on colonic compliance, tone, and pain demonstrated in this report suggest that the development of selective agents may usher in a new era in targeted therapeutics for these disorders.

The lack of effect of clonidine on gastrointestinal and colonic transit was unexpected. The sample sizes were adequate to detect a significant difference in transit, suggesting that this does not represent a type II error, except for the assessment of colonic filling at 6 h, at which the study was underpowered. Nevertheless, the overall P value for colonic filling at 6 h was 0.27 (not significant), and pairwise comparisons with placebo for 0.05 mg (P = 0.09) and 0.1 mg (P = 0.06) were not significant even without Bonferroni correction for five-dose comparisons with placebo (α = 0.01). On the other hand, it is worth noting that the literature documenting the effects of clonidine on transit showed delays in gastric emptying in the dog with doses of 30 μg/kg, a dose almost an order of magnitude (10 times) higher than the 0.3-mg dose, which was the highest permissible and tolerable dose in humans.

In summary, these in depth studies indicate that the α2 mechanisms may significantly alter the tone and compliance of the human colon and reduce its sensitivity to mechanical stimuli applied intraluminally. Our data argue for the continuation of the in depth study of the subtypes of α2-adrenergic receptors, their role in colorectal diseases, and their potential as modulators of colorectal sensory and motor functions.
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