Cholinergic stimulation enhances colonic motor activity, transit, and sensation in humans

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IN THE GASTROINTESTINAL TRACT, the effects of acetylcholine are mediated by nicotinic and muscarinic M1 receptors that are predominantly located in the myenteric plexus (13) and muscarinic M2 and M3 receptors on gastrointestinal smooth muscle (19). By increasing the availability of acetylcholine, neostigmine and other acetylcholinesterase inhibitors increase colonic motor activity in healthy subjects (2, 8, 14, 15). In clinical use, neostigmine reduces colonic distension in acute colonic pseudo-obstruction (26, 30). Putative diagnostic uses of cholinesterase inhibitors include the identification of colonic “inertia” in constipated patients, as characterized by their reduced colonic contractile responses to neostigmine (2). Conversely, the motor response to neostigmine was attenuated in myopathic, compared with neuropathic, intestinal pseudo-obstruction (3, 4). Although these observations underscore the potential therapeutic and diagnostic uses of neostigmine, the dose-response profile for its colonic motor effects is unknown. It is not known whether increased contractility induced by neostigmine is associated with accelerated colonic transit. Although increased contractile activity activates visceral afferents in vitro (20) and enhances visceral perception in humans (22), the effect of neostigmine-induced motor activity on colonic perception of distension is unknown.

On the other hand, bethanechol is a muscarinic agonist of M2 and M3 receptors (1, 13, 23). It has been used less frequently to enhance gastrointestinal contractility, despite its lack of nicotinic/cardiac effects and its prolonged duration of action after subcutaneous administration (24, 28). Abdominal cramps and diarrhea are frequent side effects of neostigmine and bethanechol (16). In this study, our specific aim was to compare the effects of neostigmine and bethanechol on colonic transit (geometric center) were compared with those of saline by scintigraphy in 21 subjects. Both drugs increased colonic phasic pressure activity, reduced rectal compliance, and enhanced urgency during rectal distension. Neostigmine also reduced colonic and rectal balloon volumes, reflecting increased tone by an average of 12% and 25% for the highest dose, respectively. Only neostigmine reduced rectal compliance, accelerated colonic transit (mean geometric center at 90 min 2.5 vs. 1.0 placebo), and increased pain perception during colonic distension. We conclude that neostigmine has more prominent colonic motor and sensory effects than bethanechol. Moreover, neostigmine induces coordinated colonic propulsion, perhaps by stimulating muscarinic M1 receptors in the myenteric plexus.

MATERIALS AND METHODS

Healthy Volunteers

The subjects provided informed consent to participate in the study protocol, which was approved by the Institutional Review Board at the Mayo Clinic. For the colonic motility studies, we recruited 30 healthy volunteers (14 male, 16 female; age 18–51 yr, mean ± SE = 33 ± 1.1 yr) by public

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volumes and manometric pressure changes in response to a noticeable deflection in the balloon volume (6). Intraballoonatory excursions during deep inspiration were accompanied by pressure, i.e., 2 mmHg above the pressure at which respira-

colonic balloon, respectively.

A clinical interview and a physical examination were performed to exclude significant systemic disease, patients who had had abdominal surgery except for appendectomy and/or cholecystectomy, and medications that could potentially interfere with bowel motility, including antidepressives. Validated screening questionnaires (a bowel disease questionnaire (31) and the hospital anxiety and depression inventory (34)) were used to exclude subjects with irritable bowel syndrome and to determine anxiety and depression scores before participation in the study.

Colonic and Rectal Motor Activity: Phasic and Tonic Motility

Method. As described previously (6), a multilumen poly-

ethylene balloon barostat-manometric assembly incorporating six manometric point transducers was positioned in the cleaned upper descending colon using flexible sigmoidoscopy and fluoroscopy (Fig. 1). An “infinitely” compliant 10-cm-long balloon with a maximum volume of 600 ml (Hefty Baggies; Mobil Chemical, Pittsford, NY) linked to an electronic rigid-
piston barostat (Mayo Rigid Barostat; Engineering Depart-
ment, Mayo Clinic, Rochester, MN) by 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 (32) recorded colonic tone. Another polyethylene balloon, 7 cm long, was placed in the rectum 5 cm from the anal verge and connected to a separate barostat to record rectal tone. The manometric sensors comprised six water-

perfused (0.4 ml/min) pneumohydraulic transducers. The first and second transducers were 5 cm oral and caudal to the colonic balloon, respectively. Sensors 2–6 were at 5-cm intervals from each other. In general, there were two trans-
ducers at each of three sites, i.e., upper descending (sensors 1 and 2), mid-descending (sensors 3 and 4), and sigmoid (sen-
sors 5 and 6) colon. The balloon was inflated to operating pressure, i.e., 2 mmHg above the pressure at which respira-
tory excursions during deep inspiration were accompanied by a noticeable deflection in the balloon volume (6). Intraballoon volumes and manometric pressure changes in response to wall contractions and relaxations were monitored continuously by these balloons. A pneumobelt was applied to the abdominal wall at the level of the lower costal margin to exclude artifact during movement and coughing.

Data analysis. Colonic and rectal motor activity were quantified using a computer program identical to those used in previous studies (5, 6). Phasic pressure activity recorded by manometric transducers was expressed as the area under the curve, transformed to log scale, which provided a near-
gaussian (“normal”) distribution. The results are reported in the original scale by inverse transformation (antilog) of the mean log values. We defined contractions that were ≥75 mmHg and propagated caudally for ≥15 cm as high-ampli-
tude propagating contractions (HAPCs). For the barostat data, the balloon volume was analyzed by a computer pro-
gram to separate baseline balloon volume (representing “tone”) from phasic volume deflections ≥10 ml over baseline volume (5, 6).

Colonic and Rectal Pressure-Volume Relationships (“Compliance”)

Method. Because colonic tone and compliance are only reproducible after a conditioning distension, balloon pressure was initially increased from 0 to 36 mmHg in 4-mmHg steps at 30-s intervals (Fig. 2; Ref. 15). Fifteen minutes later, colonic and rectal compliance were assessed separately by distending the respective barostat balloon from 0 to 36 mmHg in 2-mmHg steps at 30-s intervals.

Data analysis. The barostat balloon volume was averaged over 30 s at each pressure. Each compliance curve was summarized by a power exponential model, plotting proportionate volume (pVol = Vol/Vmax) as a function of reciprocal pressure (RP), i.e.

\[ \text{pVol} = r + \exp(-\kappa \cdot \text{RP}^\beta) \]

where RP = 1/pressure, Vmax is the maximum volume, and r is the ratio of the minimum to the maximum volume during the compliance curve. The parameter β affects the overall shape of the curve, whereas κ is essentially the change in volume as a function of 1/pressure at any given point (5, 6); Pr50 is the estimated pressure at 50% of maximum balloon volume. The parameters κ and β for each compliance curve (colonic and rectal, pre- and postdrug) were estimated using the NLIN procedure in the SAS software package (27). The estimated κ and β for each subject were used to calculate the

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**Fig. 1.** Combined colonic and rectal barostat-manometric assembly. The colonic and rectal balloons were connected to separate barostats. Manometric sensors were either proximal (sensor 1) or distal (sensors 2–6) to the colonic balloon.
pressure corresponding to half-maximum volume (Pr$_{0.5}$). The overall fit for each curve was summarized as an $R^2$ value measuring the proportion of total variation accounted for by the fitted curve.

Colonic and Rectal Sensation

Method. Colonic and rectal sensation were assessed separately by asking subjects to rate intensity of perception on visual analog scales (VAS) during balloon distensions of 8, 16, and 32 mmHg greater than the operating pressure of the corresponding segment, i.e., colon or rectum, applied in random order (6, 12). Each distension was maintained for 1 min, with an interstimulus interval of 1 min, during which the balloon was deflated to operating pressure. Subjects were asked to mark three separate 100-mm-long VAS for sensation of gas, the urge to defecate, and pain 1 min before the series of distensions and 20 s into each distension. These VAS were anchored at each end by the descriptions “unnoticeable” and “unbearable.” During assessment of sensation, verbal interaction between the subject and investigator was minimized.

Data analysis. Because the balloon volume varied during the distension, intraballoon volume was averaged over 30 s, beginning 20 s after the distension had commenced. Levels of arousal and stress, which may influence perception of colonic distension (12), were assessed immediately before assessment of colonic sensation, i.e., after drug administration. Thus two 100-mm linear VAS marked “tired to energetic” and “active to drowsy” were used to assess levels of arousal, whereas stress levels were assessed using similar scales marked “peaceful to tense” and “worried to relaxed” (12).

Drugs

In the colonic motility studies, 15 subjects were randomized to receive neostigmine and 15 subjects were randomized to receive bethanechol. Neostigmine was administered intravenously, whereas bethanechol was administered subcutaneously. The Mayo Clinic Institutional Review Board required that the investigator be aware of the drug being administered but not the specific dose. Arterial oxygen saturation, heart rate, and blood pressure were monitored throughout the study using a pulse oximeter (CO$_2$SMO; Novametrix Medical Systems, Wallingford, CT) and a telemetric monitoring device (Propac; Protocol Systems, Beaverton, OR), respectively.

Neostigmine. We studied three doses of neostigmine (Prostigmin; ICN Pharmaceuticals, Costa Mesa, CA), i.e., 0.25, 0.75, and 1.5 mg administered intravenously. The half-life of neostigmine after intravenous administration ranges from 47 to 60 min (mean 53 min) (9).

Bethanechol. Bethanechol (Urecholine; Merck, West Point, PA) was administered at a dose of 2.5, 5, or 10 mg subcutaneously, because intravenous administration is contraindicated in humans. Prior studies in humans demonstrated that these doses are safe, enhance bladder contractility (21), and encompass the dose (0.05 mg/kg sc) that increased colonic motor activity in dogs (33). The subcutaneous dose used clinically to relieve postoperative ileus and abdominal distension is 5 mg (28, 29).

Experimental Procedure

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, a plasma b-human chorionic gonadotropin pregnancy test for women of childbearing potential, and bowel preparation. Subjects drank 2–5 l of polyethylene glycol 3350 and electrolyte solution (OCL; Abbott Laboratories, Chicago, IL) until the fecal effluent became clear. After an overnight fast, left-sided colonoscopy without sedation was performed to position the colonic barostat-manometric assembly; the rectal balloon was inserted 5 cm from the anal verge. After a 30-min equilibration period, the barostat “operating pressure” was set and the experiment was started.

The experimental protocol is summarized in Fig. 2. Each subject received an intravenous injection of saline followed by a single dose of either neostigmine or bethanechol 140 min later. Colonic and rectal motor activity were recorded concurrently for 20 min before drug administration and for 30 min after drug administration, given the 10-min delay between drug administration and onset of motor effects after subcutaneous bethanechol. Colonic and rectal compliance and sensation during phasic (random) distension were measured.
separately using identical paradigms. The order of colonic and rectal testing was randomized.

**Colonic Transit Studies**

**Method.** Colonic transit was measured scintigraphically using a delayed-release, methacrylate-coated capsule containing \(^{111}\)In ion exchange pellets (Sigma, St. Louis, MO) in a separate group of 21 subjects. This method has been described in detail elsewhere (7). We initially compared the effects of intravenous neostigmine (1.5 mg) to placebo (saline) in 12 subjects who were randomized to receive either agent. Subsequently, the effects of bethanechol (5 mg sc) were compared with subcutaneous saline in nine subjects who were randomized to receive either saline (\(n = 3\)) or bethanechol (\(n = 6\)). Subjects ingested the \(^{111}\)In capsule at 6 AM on the day of the study. Scans were taken at regular intervals until the \(^{111}\)In capsule had disintegrated, releasing \(^{111}\)In activity within the ascending colon. Thereafter, these subjects were randomized to receive placebo or drug, and scans were taken every 15 min for 2 h and at 3 h.

**Data analysis.** A variable region of interest program was used to quantitate counts in 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 \(^{111}\)In counts in the \(^{99m}\)Tc window. In each scan, colonic transit was summarized as the geometric center (GC), which 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 was multiplied by its weighting factor as follows:

\[
(%AC \times 1 + %TC \times 2 + %DC \times 3 + %RS \times 4 + %stool \times 5)/100 = GC
\]

Thus a high GC implies faster colonic transit; for example, a GC of 1 implied that all isotope was in the ascending colon and a GC of 5 implied that all isotope was in the stool.

**Statistical Analysis**

The statistical analyses assessed differences in predrug parameters between agents, measured overall drug effects, and compared the effects of different doses. Phasic pressure activity, tone, and compliance (\(\kappa, \beta, Pr_{50}\)) were analyzed by an analysis of covariance (ANCOVA), with separate tests for neostigmine and bethanechol dose effects and inclusion of predrug (baseline) values as covariates. If the ANCOVA was significant, pairwise comparisons between the lowest and middle dose and the middle and highest dose of each agent were performed at an adjusted \(\alpha\)-level of 0.025 for statistical significance. All data are expressed as means ± SE unless otherwise specified.

The analysis of sensation scores (gas, urgency to defecate, and pain) over the three distending pressures (8, 16, 32 mmHg) above operating pressure was based on a repeated-measures model analysis of variance (PROC MIXED in SAS). The anxiety, depression, arousal, and stress scores were considered as potential covariates. We also considered the potential interaction between predrug and postdrug sensation scores. For the transit data, a repeated-measures analysis of variance compared GC for colonic transit at multiple times after injection between the two groups, i.e., placebo vs. neostigmine or placebo vs. bethanechol.

**RESULTS**

Thirty and twenty-one subjects participated in the colonic motility and transit studies, respectively. In the colonic motility studies, 15 subjects, of whom 8 received the highest dose of one of the drugs, developed noncardiac cholinergic side effects such as sweating, lacrimation, abdominal discomfort, or the urge to pass urine or stool. However, these side effects interrupted a comprehensive assessment of motor and sensory effects in only two subjects, one of whom received intravenous atropine to counteract severe abdominal cramps after neostigmine (1.5 mg); the other subject experienced vasovagal syncope after bethanechol (10 mg). Technical problems related to the barostat-manometric assembly and/or computer data management prevented assessment of 1) colonic balloon volume in three subjects who received 0.25 mg neostigmine, 2.5 mg bethanechol, and 10 mg bethanechol, respectively, and 2) rectal balloon volume only at the operating pressure in two subjects who received 5 mg bethanechol. In the colonic transit studies, all six patients who received neostigmine (1.5 mg iv) and five of six subjects who received bethanechol (5 mg sc) experienced one or more cholinergic side effects, including dizziness, salivation, facial flushing/chills, diaphoresis, urinary urgency, abdominal cramps, diarrhea, and transient syncope. These side effects did not prevent assessments of transit from being completed.

**Effect on Colonic Phasic Activity**

Colonic phasic pressure activity before drug administration was not significantly different among drug dose groups but was usually higher in the sigmoid than in the descending colon. Figures 3 and 4 demonstrate that both drugs increased phasic manometric pressures in the upper descending (sensor 1), mid-descending (sensor 3), and sigmoid (sensor 6) colon. Figure 4 summarizes the postdrug values, adjusted for differences in predrug values among groups. Neostigmine
had significant dose effects on phasic activity; it increased phasic activity in the upper descending (P = 0.004), mid-descending (P = 0.025), and sigmoid (P = 0.015) colons. Pairwise comparisons revealed significant (P = 0.016) differences between 0.75 mg and 0.25 mg, but not between 0.75 mg and 1.5 mg, neostigmine in the upper descending colon. Bethanechol increased colonic phasic pressure activity, but dose effects were not statistically significant.

**Effect on Colonic and Rectal Balloon Volume at Operating Pressure**

Mean barostat operating pressures were similar in the descending colon and rectum [i.e., 9.1 ± 0.4 and 10.4 ± 0.4 (mean ± SE) mmHg, respectively]. Predrug values for colonic and rectal balloon volume were similar among the drug dose groups and significantly (P < 0.001) associated with postdrug values. Neostigmine (0.75 and 1.5 mg) reduced colonic balloon volume, suggesting increased colonic tone. Figure 3 shows that the reduction in balloon volume frequently preceded the augmentation of phasic activity recorded by manometric sensors. After adjustment for differences in predrug values, neostigmine, but not bethanechol, had significant overall dose effects on colonic (P < 0.01) and rectal (P < 0.05) balloon volume (Table 1). In the colon, the 0.75 mg dose was significantly different from the 0.25 mg dose (P = 0.006) but not from the 1.5 mg dose. In the rectum, the 0.75 mg dose was different (P = 0.01) from the 1.5 mg dose, but the pairwise comparison of 0.25 mg and 0.75 mg was not significant (P = 0.43).

**Effect on Colonic and Rectal Pressure-Volume Relationships (Compliance)**

Colonic and rectal pressure-volume relationships were summarized by a power exponential model, defined by the parameters \( k \), which represents the instantaneous slope, and \( \beta \), reflecting the overall shape. The median (range) \( R^2 \) value for the fit to the power exponential model was 97.95% (88.6–99.8%) in the colon and 99% (93.4–99.9%) in the rectum. Predrug values for \( k \) and \( \beta \) were not statistically different among drug dose groups. Examples of observed and fitted compliance curves are shown in Fig. 5, and the estimated parameters \( k \), \( \beta \), and \( \text{Pr}_{1/2} \) for the individual doses are summarized in Table 2. Neostigmine (P = 0.03) and bethanechol (P = 0.02) significantly reduced rectal compliance, increasing balloon pressure (Pr\(_{1/2}\)) required to reach half-maximum volume. Neostigmine also increased (P = 0.02) the \( \text{Pr}_{1/2} \) for colonic pressure-volume relationships, indicating reduced colonic compliance. After adjustment for baseline differences between groups, pairwise comparisons of \( \text{Pr}_{1/2} \) between 0.25 mg and 0.75 mg neostigmine and between 0.75 mg and 1.5 mg neostigmine were not statistically different. Neostigmine and bethanechol did not affect maximum recorded balloon volume during colonic or rectal pressure-volume curves.

**Effect on Sensory Perception of Colonic and Rectal Distension**

By repeated-measures analysis of variance, neostigmine significantly increased pain sensation during colonic distension (P = 0.006, dose effect), whereas both neostigmine (P = 0.003, dose effect) and bethanechol (P = 0.03, dose effect) increased the sensation of ur-
gency during rectal distension (Table 3). Neither drug altered the sensation of gas during colonic or rectal balloon distension (data not shown). Table 3 shows that the neostigmine effect was most noticeable for the 1.5 mg dose, at which subjects noted high ratings for colonic pain and rectal urgency even at operating pressure, i.e., before phasic distensions. In contrast, betanechol had relatively modest effects on sensory scores before distension. Moreover, repeated-measures analysis of variance also revealed a significant inter-

Table 2. Effect of neostigmine and betanechol on colonic and rectal compliance

<table>
<thead>
<tr>
<th>Drug dose, mg</th>
<th>Colon</th>
<th></th>
<th>Rectum</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\kappa$</td>
<td>$\beta$</td>
<td>$Pr_{1/2}$</td>
<td>$\kappa$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Pre-Neo ($n = 13/14$)*</td>
<td>13.7 ± 0.9</td>
<td>1.4 ± 0.1</td>
<td>11.6 ± 0.9</td>
<td>12.6 ± 0.7</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Neo 0.25 ($n = 4/5$)*</td>
<td>14.9 ± 0.8</td>
<td>1.6 ± 0.3</td>
<td>12.2 ± 2.2</td>
<td>14.2 ± 0.8</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Neo 0.75 ($n = 5$)</td>
<td>16.5 ± 1.0</td>
<td>2.4 ± 0.3</td>
<td>17.1 ± 1.6</td>
<td>15.1 ± 1.7</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Neo 1.5 ($n = 4$)</td>
<td>16.6 ± 2.2</td>
<td>2.3 ± 0.7</td>
<td>16.0 ± 3.2</td>
<td>16.6 ± 0.8</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Pre-Beth ($n = 13/14$)*</td>
<td>14.3 ± 1.2</td>
<td>1.5 ± 0.1</td>
<td>12.5 ± 0.9</td>
<td>14.9 ± 1.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Beth 2.5 ($n = 4/5$)*</td>
<td>15.9 ± 1.6</td>
<td>2.1 ± 0.4</td>
<td>15.4 ± 1.5</td>
<td>15.3 ± 2.3</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Beth 5.0 ($n = 5$)</td>
<td>16.5 ± 1.8</td>
<td>1.6 ± 0.1</td>
<td>15.2 ± 2.0</td>
<td>17.7 ± 1.7</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Beth 10 ($n = 4$)</td>
<td>14.5 ± 0.7</td>
<td>1.5 ± 0.1</td>
<td>12.5 ± 0.8</td>
<td>18.5 ± 3.3</td>
<td>2.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of animals. $Pr_{1/2}$, pressure corresponding to half-maximum volume during the compliance curve; $\kappa$, $\beta$, compliance curve-parameters (see text). The postdrug values are adjusted for baseline differences in the corresponding predrug values among dosages. Overall, Neo reduced colonic and rectal compliance ($\uparrow \kappa$, $\uparrow \beta$, $\uparrow Pr_{1/2}$), whereas Beth reduced rectal compliance; dose-response effects were not statistically significant. *One of the subjects did not have colonic compliance.
Table 3. Effect of neostigmine and bethanechol on colonic and rectal sensation at 4 levels of balloon distension

<table>
<thead>
<tr>
<th>Drug dose, mg</th>
<th>0</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>0</th>
<th>8</th>
<th>16</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Neo 0.25</td>
<td>14.0 ± 11.6</td>
<td>26.6 ± 14.6</td>
<td>23.0 ± 15.9</td>
<td>54.8 ± 14.4</td>
<td>32.6 ± 10.5</td>
<td>40.6 ± 17.2</td>
<td>61.2 ± 13.3</td>
<td>69.0 ± 11.0</td>
</tr>
<tr>
<td>Neo 0.25</td>
<td>10.0 ± 5.0</td>
<td>34.2 ± 12.9</td>
<td>30 ± 13.3</td>
<td>48.4 ± 9.7</td>
<td>37.2 ± 21.1</td>
<td>33.6 ± 16.2</td>
<td>68.8 ± 14.6</td>
<td>81.0 ± 6.9</td>
</tr>
<tr>
<td>Pre-Neo 0.75</td>
<td>36.4 ± 13.2</td>
<td>24.5 ± 9.5</td>
<td>40.4 ± 12.0</td>
<td>46.2 ± 13.7</td>
<td>29.4 ± 10.2</td>
<td>31.2 ± 13.6</td>
<td>50.4 ± 6.0</td>
<td>72.6 ± 2.2</td>
</tr>
<tr>
<td>Neo 0.75</td>
<td>32.6 ± 11.8</td>
<td>28.6 ± 7.7</td>
<td>27.4 ± 10.4</td>
<td>31.4 ± 11.2</td>
<td>39.2 ± 7.7</td>
<td>42.6 ± 11.1</td>
<td>55.4 ± 9.3</td>
<td>76.6 ± 3.7</td>
</tr>
<tr>
<td>Pre-Neo 1.5</td>
<td>(n = 4)</td>
<td>28.8 ± 3.9</td>
<td>29.3 ± 5.2</td>
<td>26.5 ± 5.6</td>
<td>46.0 ± 9.9</td>
<td>17.5 ± 9.9</td>
<td>36.5 ± 12.3</td>
<td>53.5 ± 15.9</td>
</tr>
<tr>
<td>Neo 1.5</td>
<td>(n = 4)</td>
<td>60.3 ± 13.8</td>
<td>57.5 ± 16.7</td>
<td>59.5 ± 13.9</td>
<td>71.0 ± 14.8</td>
<td>62.5 ± 10.4</td>
<td>66.8 ± 8.5</td>
<td>83.5 ± 5.5</td>
</tr>
<tr>
<td>Pre-Beth 2.5</td>
<td>36.8 ± 17.5</td>
<td>36.0 ± 17.9</td>
<td>32 ± 18.1</td>
<td>53.0 ± 17.1</td>
<td>40.4 ± 16.9</td>
<td>39.8 ± 14.4</td>
<td>46.0 ± 9.1</td>
<td>80.4 ± 8.0</td>
</tr>
<tr>
<td>Beth 2.5</td>
<td>44.5 ± 19.4</td>
<td>39.8 ± 18.8</td>
<td>49.3 ± 19.5</td>
<td>64.8 ± 21.7</td>
<td>45.8 ± 13.3</td>
<td>46.2 ± 12.2</td>
<td>70.0 ± 9.4</td>
<td>77.8 ± 9.3</td>
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<tr>
<td>Pre-Beth 5.0</td>
<td>11.4 ± 3.5</td>
<td>19.4 ± 9.4</td>
<td>18.8 ± 7.7</td>
<td>25.6 ± 5.1</td>
<td>27.2 ± 8.7</td>
<td>33.2 ± 16.9</td>
<td>52.6 ± 14.0</td>
<td>67.5 ± 11.0</td>
</tr>
<tr>
<td>Beth 5.0</td>
<td>21.4 ± 13.1</td>
<td>20.6 ± 9.4</td>
<td>21.0 ± 10</td>
<td>35.0 ± 13.0</td>
<td>22.8 ± 13.9</td>
<td>43.0 ± 16.8</td>
<td>52.6 ± 16.2</td>
<td>67.6 ± 15.3</td>
</tr>
<tr>
<td>Pre-Beth 10.0</td>
<td>(n = 4)</td>
<td>14.4 ± 8.1</td>
<td>16.6 ± 4.0</td>
<td>15.6 ± 5.6</td>
<td>32.4 ± 11.2</td>
<td>27.2 ± 7.2</td>
<td>24.2 ± 5.5</td>
<td>52.4 ± 8.2</td>
</tr>
<tr>
<td>Beth 10.0</td>
<td>(n = 4)</td>
<td>36.5 ± 19.0</td>
<td>39.5 ± 15.3</td>
<td>37.3 ± 15.5</td>
<td>44.8 ± 17.8</td>
<td>18.8 ± 10.9</td>
<td>15.0 ± 4.2</td>
<td>51.8 ± 11.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects (5 unless stated otherwise). Pre-Neo and Pre-Beth refer to predrug sensation scores. The column heading “0” refers to the sensation score before distension (i.e., at “operating” pressure); 8, 16, and 32 refer to balloon distension by pressures 8, 16, and 32 mmHg above operating pressure, respectively.

action between predistension sensation scores and perception of colonic pain (P = 0.01) and rectal urgency (P = 0.03). Thus neostigmine’s effects on colonic sensation as assessed by low (P = 0.01, 8 mmHg) and high (P = 0.02, 32 mmHg) distending pressures were significant when predrug pain sensation scores were ≤ 25/100. Similarly, dose effects of neostigmine and bethanechol on the sensation of urgency during rectal distension were also noted for low to moderate (≤ 35/100) predrug urgency sensation scores at a distending pressure of 16 mmHg (P = 0.01 and P = 0.02, respectively). On the other hand, when scores for pain or urgency before drug administration were high, neostigmine and bethanechol did not have significant dose effects on colonic or rectal sensation, respectively.

**Effect on Colonic Transit**

GC for colonic transit over the 3-h time period was comparable for placebo vs. bethanechol (P = not significant [ns]) but differed between placebo and neostigmine (P < 0.01). Neostigmine, but not bethanechol, significantly accelerated colonic transit (Figs. 6 and 7) by 45 min after administration. For neostigmine, there was no further isotope migration after 90 min, at which point the mean GC was 2.5, i.e., in the mid-descending colon.

**Effect on HAPCs**

HAPCs occurred frequently in the 30-min period after the drug was given (Fig. 8). Thus HAPCs were observed in two subjects before drug administration and in six subjects after drug administration (P = ns). Three of six subjects who received 1.5 mg of neostigmine had 1, 6, and 16 HAPCs. Another three subjects developed a single HACP, either after neostigmine (0.75 mg) or bethanechol (2.5 and 5 mg).

Fig. 6. Examples of neostigmine (A) and bethanechol (B) effects on colonic transit in individual subjects. Note rapid aboral migration of $^{111}$In isotope in scans taken 15, 30, and 120 min after injection of neostigmine (1.5 mg iv) but not bethanechol.
DISCUSSION

These experiments compared the effects of a cholinesterase inhibitor (neostigmine) and a muscarinic cholinergic agonist (bethanechol) on colonic and rectal motor and sensory function in healthy subjects. Both drugs increased colonic phasic pressure activity, reduced rectal compliance, and enhanced urgency during rectal distension. However, only neostigmine reduced colonic and rectal balloon volumes and colonic compliance, suggesting that this agent increased tone. Moreover, only neostigmine accelerated colonic transit and increased pain perception during colonic distension.

The differential effects of neostigmine and bethanechol on colonic transit have practical clinical implications, and they should be attributable to differences between the muscarinic receptor subtypes stimulated by these agents. In the colonic transit studies, the rapid emptying of radioisotope from the proximal to the distal colon after neostigmine (1.5 mg iv) implies that mass movements are induced by HAPCs (10). A higher dose of neostigmine (i.e., 30 \( \mu g/kg \)) invariably induced HAPCs in dogs (17); we observed cholinergic side effects in healthy subjects who received 1.5 mg neostigmine intravenously. These findings and anecdotal clinical observations suggest that neostigmine at an intravenous dose of 1.5 mg may be as effective, and possibly safer, than the recommended intravenous dose of 2 mg in elderly patients with acute colonic pseudo-obstruction (26). In contrast to neostigmine, an
HAPC was observed in only 1 of the 10 subjects who received bethanechol (5 or 10 mg). These findings support the hypothesis that bethanechol, a selective muscarinic M₃ receptor agonist, contracts muscle but does not induce coordination, which is necessary to accelerate colonic transit (13). The muscarinic receptors that induce coordinated activity are perhaps more likely located on intramural neurons and are of the M₁ type (13).

We studied the effects of the 5 mg dose of bethanechol on colonic transit, because this dose increased colonic phasic activity and rectal tone in the motility studies. In previous studies, this dose also increased gastric antral activity and urinary bladder contractility and reduced postoperative abdominal distension in humans (11, 25, 28, 29). We did not assess the effects of 10 mg bethanechol on colonic transit because this dose did not induce colonic HAPCs but did cause significant cholinergic side effects, including syncope in one subject, during the motility studies. Moreover, in contrast to neostigmine, bethanechol did not have significant dose effects on any parameter of colonic motor activity or sensation. These data are consistent with previous studies that failed to demonstrate a dose-dependent effect for bethanechol on urinary bladder contractility (11) or antroduodenal motor activity in humans (18).

Another difference between bethanechol and neostigmine was that bethanechol significantly increased colonic phasic activity and reduced rectal compliance but did not significantly affect colonic tone or compliance. These results suggest that bethanechol has a lesser effect on colonic tone than does neostigmine, even at doses that have similar effects on colonic phasic activity. Although a type II error cannot be completely excluded, it is unlikely that a clinically significant effect of bethanechol on colonic tone was missed. In addition, 5 mg (n = 5) and 10 mg (n = 4) bethanechol did not affect colonic compliance, whereas neostigmine increased tone and reduced compliance in the colon and rectum. These observations also support the concept that pressure-volume relationships can be altered pharmacologically by agents that modulate muscle tone, particularly at low and medium pressures but not at high pressures (6). Thus increased tone was manifested by a shift in the pressure-volume curve to the right at pressures in the low and midrange, but not at highest imposed pressures; maximum balloon volume was not affected by neostigmine or bethanechol.

Three observations may be made regarding drug effects on perception of visceral distension. First, from the perspective of visceral hypersensitivity, our observations add credence to earlier data that suggest that increased rectal tone is associated with enhanced rectal perception during balloon distension. However, it is still unsettled as to whether sensory effects are consequent to, or independent of, effects on tone (22). Second, both neostigmine and bethanechol increased the sensation of urgency but not of gas or pain during rectal distension. This suggests that thresholds for the desire to defecate (urgency) may be preferable to the threshold of “pain” when assessing for drug effects on sensation during rectal distension (15). Finally, our results underscore the importance of factoring predrug and postdrug sensation scores at operating pressure, i.e., before distension, in analyzing drug effects on sensation, even in healthy subjects (12). A “ceiling” effect may have masked significant drug effects on sensation when predrug sensation scores or postdrug sensation scores at operating pressure are high, e.g., after intravenous 1.5 mg neostigmine, even before balloon distension.

In summary, the cholinesterase inhibitor neostigmine, which stimulates muscarinic M₁/M₂/M₃ receptors, has more prominent colonic motor and sensory effects than the muscarinic M₃ cholinergic agonist bethanechol. Moreover, in contrast to bethanechol, neostigmine (1.5 mg iv) accelerated colonic transit. These results provide the rationale for the beneficial effects of neostigmine in acute colonic pseudo-obstruction and suggest that stimulation of muscarinic M₁ receptors in the myenteric plexus is necessary for coordinated colonic propulsion.

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