

Control of muscle tone in the human colon

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Abstract

Human colonic muscle tone varies diurnally and postprandially in predictable ways. Increased tone reduces the capacity of the colon to store contents after a meal, whereas increased distensibility (lesser tone) during sleep enlarges the storage capabilities and may slow transit. We tested the hypothesis that antidiarrhoeal drugs would also alter tone which, in turn, might reduce diarrhoea by facilitating the storage and salvage of fluids. Using a colonic barostat to create low pressure, isobaric colonic distension in healthy volunteers, we found that intravenous atropine (0.01 mg/kg) relaxed the colon during fasting, reduced the postprandial increase in tone, and enhanced relaxation in the late (1-2 hour) postprandial period. Intravenous morphine (0.1 mg/kg) caused variable effects soon after injection but, in fasting subjects, the descending colon relaxed 70-90 minutes after morphine. These changes in colonic motility were not always obvious by conventional manometric recording. Colonic distensibility is increased by antidiarrhoeal drugs and this effect may contribute to their efficacy in slowing colonic transit and augmenting absorption.

Human colonic muscle exhibits rhythmic diurnal tone, being maximal after meals and least during nocturnal sleep.¹ These fluctuations are consistent with the general hypothesis that the colon relaxes to accept materials arriving from above and constricts in order to move contents distally. The propensity of the colon to relax also suggests a mechanism that might underlie colonic dilatation, this being a feature of several pathophysiological states ('megacolons').

We wished to investigate the pharmacological control of colonic muscle tone by those drugs known to lessen diarrhoea. Thus, opiate narcotics reduce the volume of diarrhoea but the mechanisms of this effect are incompletely understood.^{2,3} Drug induced changes in the frequency, amplitude, and rhythmicity of phasic contractions in the small intestine seem to be important in slowing intestinal transit.^{2,3} An alternative but little investigated explanation is that opiates cause receptive relaxation of the colon, thereby increasing its reservoir capacity and, thus, its ability to retain contents for absorption of fluid and electrolytes.⁴ Our previous scintigraphic studies documented that morphine relaxed the ascending colon and slowed the onset of the diarrhoea induced by the infusion of oleic acid into the lumen.⁴ Another association of colonic hypotonia is the clinical recommendation that opiates be used cautiously in the presence of colonic inflammation because of their incrimination in acute colonic dilatation ('toxic megacolon').⁵ Our hypothesis was that

opiates would reduce colonic tone, causing the colon to become highly compliant and more easily stretched. Muscarinic blockers, though used infrequently as antidiarrhoeal drugs, are constipating; these agents should also facilitate colonic relaxation.

The barostat, which was developed and validated by Malagelada and Azpiroz^{6,7} in our laboratory, monitors continuously, at a constant pressure, the volume of air within a highly compliant bag placed within a segment of bowel. We have already applied this methodology to the human¹ and canine⁸ colons; changes in colonic tone, as reflected by changes in volume of the barostat bag, were recorded precisely.^{1,8} Using the colonic barostat in man we aimed to test the hypothesis that opiate and anticholinergic drugs would reduce colonic muscle tone and increase the capacity of the colon, facilitating its function as a reservoir.

Methods

SUBJECTS

Fourteen healthy volunteers (three women and 11 men; ages 20-38 years) were recruited by public advertisement. They gave informed consent to participate in experimental protocols that had been approved by the Institutional Review Board and Radiation Control Committee of the Mayo Clinic. All were required to have a normal physical examination before being accepted into the study and none was taking medication other than oral contraceptives. All had a normal bowel habit of between 3 stools/day and 3 stools/week and none had any symptoms of gastrointestinal disease. Subjects were admitted to the Clinical Research Center and prepared for study by ingesting a colonic lavage solution composed of polyethylene glycol and electrolytes (Oral Colonic Lavage Solution, Abbott Laboratories, Chicago, IL) until the faecal effluent was clear liquid, free of particulate matter.

PROCEDURES

Fibreoptic colonoscopy was performed to the caecum, or as far as was limited by minimal discomfort. No retained solid matter was observed in the colon of any subject. Premedications for colonoscopy were the minimal doses of intravenous midazolam (2-5 mg) or pethidine hydrochloride (50-100 mg), or both, necessary to maintain acceptable levels of discomfort. A soft teflon coated guidewire was inserted into the colon under fluoroscopic control and the colonoscope was withdrawn. Colonic catheters were then inserted over the guidewire to the desired colonic segment (see below). All subjects recovered rapidly from the sedation and were alert at the start of the experiments. The positions of

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the colonic catheter and barostat balloon were checked fluoroscopically before each experiment.

RECORDING ASSEMBLY

The multilumen catheter comprised lumens of the 0.125 inch ID and 0.06 inch ID to operate the barostat balloon, a lumen for the guidewire, and six capillary lumens for water perfused, low compliance side hole manometry.¹ The barostat balloon was a 10 cm long, highly compliant plastic bag fixed at both ends to the catheter. Intraballoon pressure was measured by a strain gauge open to the balloon's interior through a lumen of the colonic catheter. The strain gauge was linked by an electronic relay to a bellows pump that moved air into or out of the balloon at a flow rate of 23 ml/second. The pump was activated with a lag time of <5 milliseconds, when the balloon pressure deviated by more than 0.25 mm Hg from the preset pressure.¹

The preset pressures in the balloon were chosen during fasting observations before each experiment and were individualised to a constant level between 8 and 13 mm Hg. The pressures selected were the minimal ones necessary to record regular variations in balloon volume during respiratory movements which changed intra-abdominal pressure. The barostat system was checked for air leaks before and after the experiments but was consistently airtight.

Compression of air and compliance in the closed barostat system created a near linear artifactual increase in balloon volume of 3–5 ml/mm Hg. However, this artifact was relatively constant at the pressures imposed upon the system. Our approach gave priority to achieving comparable intraluminal distending pressures for each individual at the beginning of each experiment rather than starting each experiment with the same volume in the balloon. Thus, the actual basal volumes varied between individuals but the intraballoon pressure remained constant for each person.

EXPERIMENTAL DESIGN

Effects of a cholinergic agonist and antagonist

Colonoscopy and colonic catheterisation were performed at 8.00 am. In eight volunteers (two

women and six men; aged 21–38 years), basal volumes of the balloon in the proximal descending colon and intraluminal pressures of the descending and sigmoid colons were monitored from 12 noon. One hour later, a randomised, placebo controlled, intravenous dose of edrophonium (0.12 mg/kg or saline) was injected over five minutes. After monitoring for a further two hours, a randomised, placebo controlled intravenous injection of atropine (0.01 mg/kg or saline) was given over five minutes. Twenty minutes later all subjects consumed a standardised 750 ml, 1000 kcal (53% fat, 35% carbohydrate, 12% protein) liquid meal. Subjects watched television throughout, so that distractions were minimised and sleep was prevented.

Effects of morphine

The effects of opiates were approached in three ways. Our aims were to test (1) the effect of an injection or morphine during fasting; (2) the effect of morphine after muscarinic blockade, and (3) the effect of morphine in the postprandial period. In eight volunteers (three women and five men; aged 20–38 years), morphine (0.1 mg/kg) was injected intravenously 20 minutes after a randomised intravenous injection of atropine (0.01 mg/kg) or a saline placebo. In these experiments, the volume of the proximal descending colon and intraluminal pressures of the descending and sigmoid colons were monitored for one hour before the atropine/saline injection and for two hours after the injection of morphine.

In a separate series of experiments, a randomised, placebo controlled injection of intravenous morphine (0.1 mg/kg) was given over five minutes to eight subjects (all men, ages 26–38 years) two hours after the standardised meal. Colonic tone was monitored in the ascending (n=3), transverse (n=2), and descending colons (n=3) to determine whether morphine could reverse the increase in tone that follows a meal. Recordings continued for two hours after the injection.

ANALYSIS

Data storage and analysis

Pressures and volumes in the barostat balloon, pressure fluctuations registered by manometry, respiratory movement as recorded by a pneumobelt, and experimental interventions were recorded as analogue signals on Honeywell paper recorders. The analogue signal was sampled at 4 Hz, digitised, entered into a computer (Digital Equipment Corporation Microvax System), and stored on magnetic hard disc and tape for later analysis. All analyses were done by computer. The stored digitised data were scaled to match the experimental calibration and processed sequentially from the start of the recording. Barostat balloon volumes were averaged over each minute ('barostat minute volumes'). Using a modified Vaxlab peak finding and filtering program (Digital Equipment Corporation), manometric data were cleared of movement artifact by computerised deletion of small pressure waves

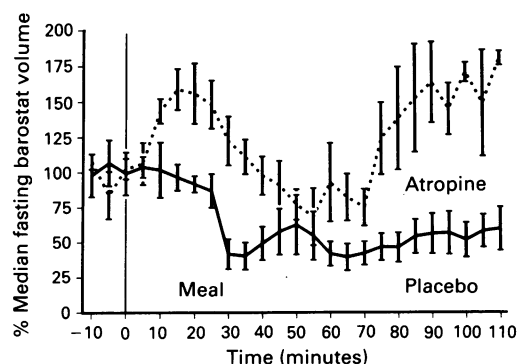


Figure 1: Group (n=8) mean (SEM) balloon volumes showing effect of intravenous atropine (0 minutes) and eating (20 minutes) on median balloon volumes; basal preprandial volumes were assigned a value of 100%. Note that the postprandial increase in tone was present after atropine.

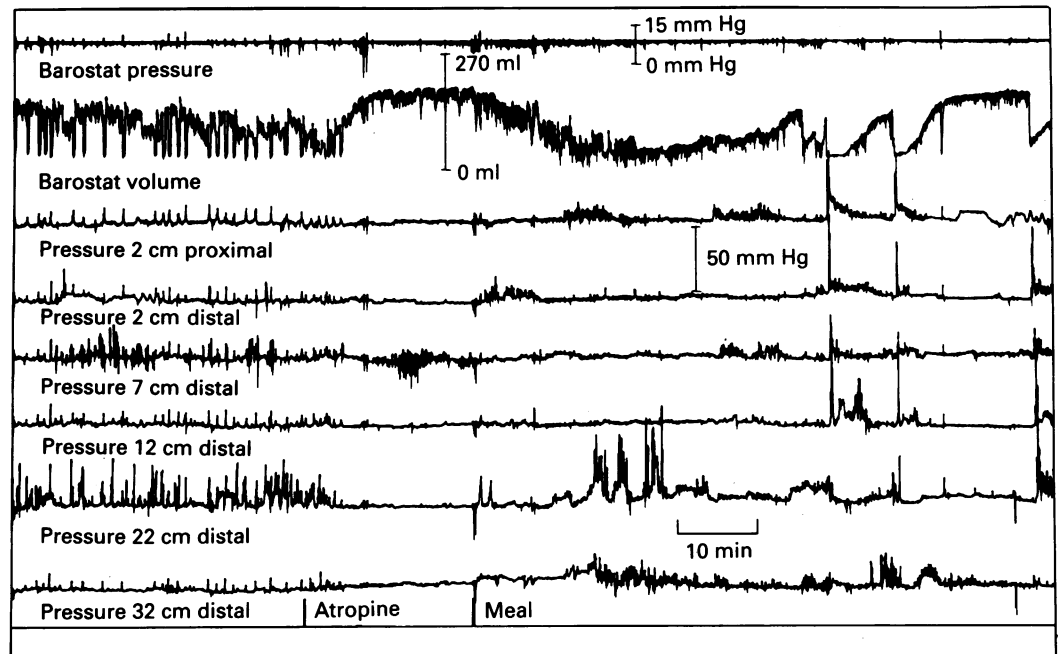


Figure 2: Barostat manometry recording from descending colon. The uppermost tracing is of the constant pressure within the balloon and the next recording is of the volume of air in the balloon. Intravenous atropine (first slash) reduced colonic tone and balloon volume increased; eating (meal, second slash) increased tone and reduced the volume of the balloon. Six manometric tracings orad (proximal) or caudad (distal) to the balloon are also shown.

(less than 4 mm Hg in magnitude or less than four seconds' duration) that occurred simultaneously over multiple manometric recording sites. The manometric data were then passed through a polynomial filter to remove minimal pressure variations (2–6 mm Hg); the number of pressure waves (peaks) was counted and the sum of the peak amplitudes calculated for each minute. Phasic pressure activity recorded from each manometry site was summarised as a motility (pressure) index for each minute using the following formula:

$$\text{In} [\text{number of peaks} \times \text{sum of peak amplitudes} + 1].$$

Phasic pressure waves were interpreted as being caused by phasic contractions of colonic muscle.

Barostat minute volumes and motility indices were electronically transferred to computerised worksheets (Clinfo, NIH, Bethesda). In each experiment, the median barostat minute volume in the basal fasting period preceding the experimental interventions was taken to represent the basal volume of the colonic segment occupied by the barostat balloon. As balloon volumes varied considerably between individuals, the actual barostat minute volumes in each experiment were expressed as a percentage or proportion of

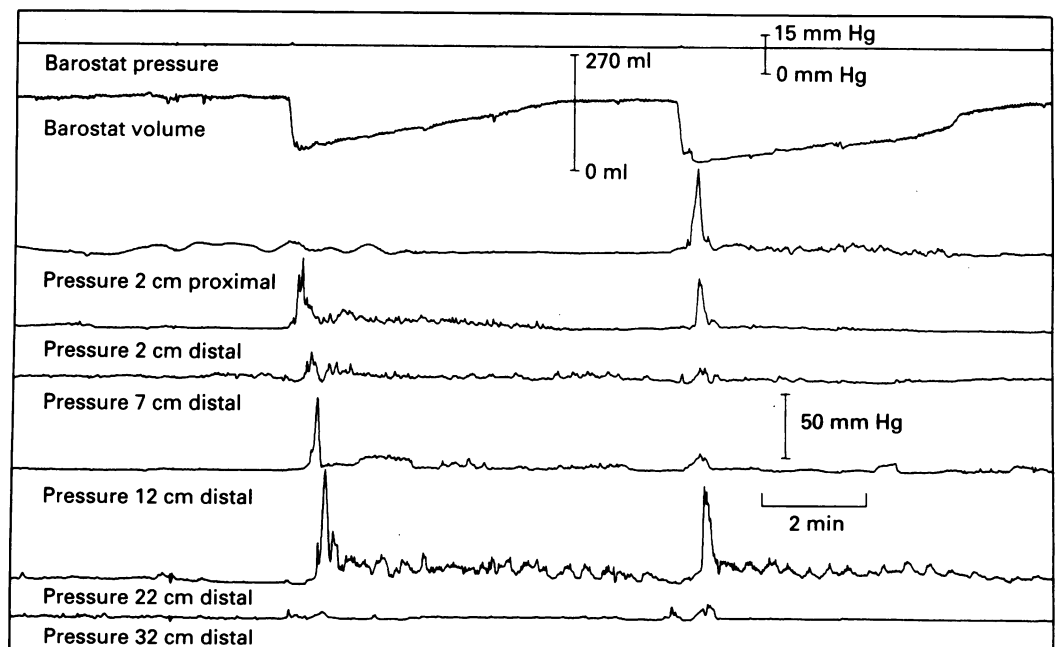


Figure 3: Barostat manometry recordings illustrating the decrease in balloon volumes (second tracing from top), and slow return to basal values, when a giant migrating contraction was recorded manometrically from side holes proximal and distal to the balloon.

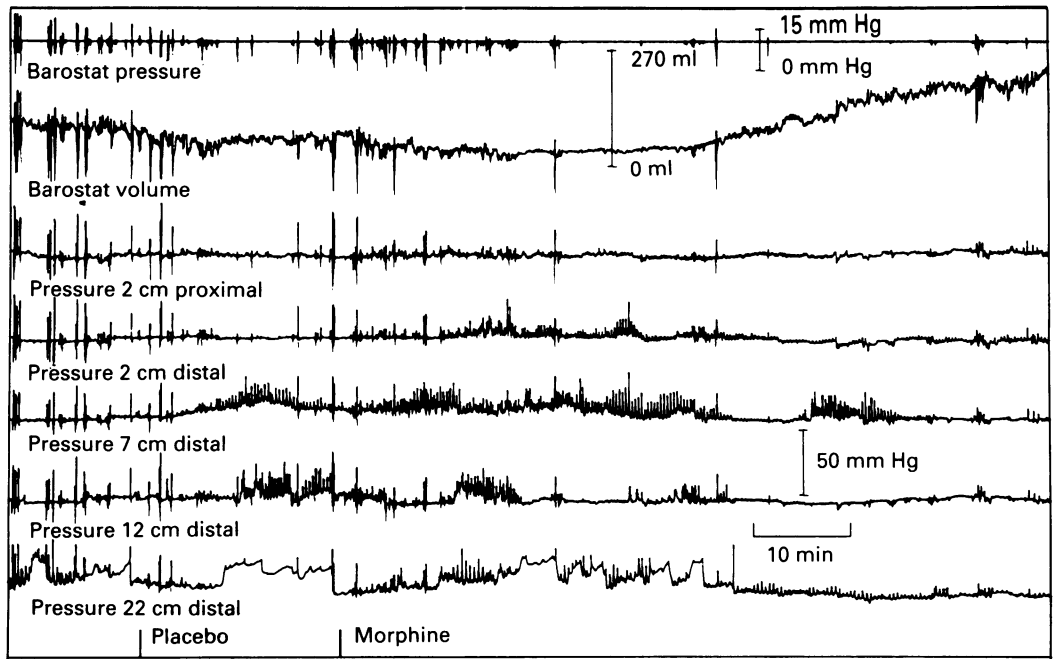


Figure 4: Barostat manometry recordings from descending colon illustrating slow decrease in tone (increase in balloon volume, second channel) that begun approximately 40 minutes after an injection of morphine.

the median basal volume (proportional minute volumes). This approach minimised effects of compression artifacts and variations in colonic anatomy on the results.

Statistical comparisons before and after experimental interventions were performed with paired, two tailed *t* tests; the responses to placebo and active drug were compared using unpaired, two tailed *t* tests. Values quoted in the text are mean (SEM).

Results

Volumes recorded by the barostat during fasting were essentially stable (Fig 1). Placebo injections produced no significant change in barostat balloon volumes.

EFFECTS OF ATROPINE

Atropine increased fasting balloon volumes (Figs 1 and 2); it caused a 61 (10)% increase in volume 10 minutes after injection ($p < 0.01$). Muscarinic blockade also reduced slightly the absolute increase in colonic tone that occurs postprandially and decreased the late (60–80 minutes) increase of postprandial tone ($p < 0.01$; Figs 1 and 2).

Giant migrating contractions (GMC), which were sometimes recorded after the atropine-meal sequence, caused the balloon to empty rapidly. These episodes were followed by slow refilling of the balloon (Fig 3). Thus, atropine, which reduced phasic contractile activity appreciably throughout the descending and sigmoid colon, did not inhibit the generation of GMCs postprandially.

EFFECTS OF MORPHINE

During fasting, injections of morphine produced variable responses. In most subjects there was no initial response of the barostat to morphine but

colonic relaxation began 40–60 minutes after the drug (Figs 4 and 5). Thus, 70–90 minutes after morphine, balloon volumes in the descending colon of fasting subjects were 81 (18)% greater than preinjection volumes ($p < 0.05$; Fig 5). All of the subjects who received morphine became mildly drowsy but they were prevented from sleeping as much as possible. When atropine was given before the opiate, morphine did not induce any additional colonic relaxation (compare Figs 2 and 5).

In the postprandial period, when colonic muscle tone was maximal, morphine caused a transient but inconsistent reduction of colonic tone. This relaxation lasted 10–20 minutes and it occurred soon after injection. In one subject, who also became mildly hypotensive, relaxation of the ascending colon was very noticeable (Fig 6).

EFFECTS OF EDROPHONIUM

Edrophonium is an anticholinesterase agent that was tested for its ability to increase cholinergic transmission. The drug induced a variable increase in repetitive, phasic contractions of the descending and sigmoid colons, but it had no consistent effect on colonic muscle tone.

MOTILITY INDICES

Despite the presence of an inflated barostat balloon in the colon, the normal gradient of phasic contractile activity between the descending and sigmoid colon was preserved. Phasic contractile activity of the sigmoid colon was usually greater than that of the descending colon.¹

Discussion

Using the colonic barostat, we have extended earlier observations on colonic tone in man¹ and

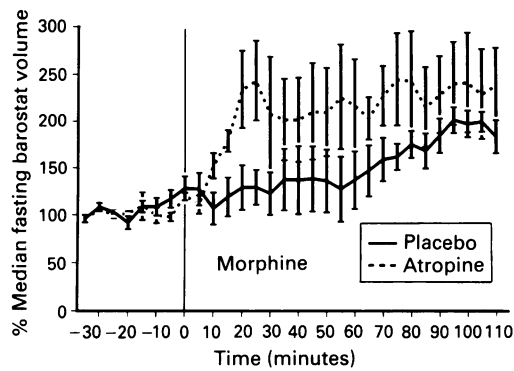


Figure 5: Group ($n=8$) mean (SEM) balloon volumes showing effect of an injection of morphine on colonic tone. An intravenous injection of atropine or placebo was given at 20 minutes and morphine at 0 minutes.

the dog.⁸ The present studies show that muscarinic blockade decreased muscle tone during fasting, blunted the expected postprandial increase in tone, and enhanced late postprandial relaxation. The effects of morphine were less consistent but the drug relaxed the proximal colon in some volunteers and was consistently associated with relaxation of the descending colon more than an hour after injection. During long periods of observation, fasting or after placebo injections, comparable episodes of relaxation were not observed. Taken together, these responses suggest that an increase in the colon's capacity to act as a reservoir is an important component of the response to these two anti-diarrhoeal agents.

Late relaxation after morphine was an unexpected finding and is difficult to explain on a pharmacological basis. The best known effects of morphine on the human colon are slowing of transit and stimulation of repetitive phasic contractions in the sigmoid colon.^{9,10} However, morphine also prevented the constriction of the proximal colon caused by an intraluminal in-

fusion of oleic acid.⁴ Indeed, the observations of Kamath *et al.*⁴ prompted the present studies. It is of note that when morphine was given postprandially, when colonic tone was raised, relaxation was usually seen. Relaxation by opiates could be mediated by indirect inhibition of cholinergic innervation of colonic smooth muscle.¹¹ It is of note, however, that there was no consistent relaxation soon after the injection, when tissue morphine concentrations must have been highest, nor did morphine increase the relaxation induced by atropine. There are theoretical arguments against morphine acting locally. Morphine is a mu receptor agonist, while opioid neurotransmission in the colon seems to be mainly enkephalinergic and to be mediated by the delta receptor.¹² Withdrawal from narcotic addiction is often accompanied by severe diarrhoea, however, and some observers have reported reversal of idiopathic constipation by naloxone, a specific opioid antagonist.¹³ Moreover, tissue morphine concentrations correlated with its effects on intestinal transit in the rat.¹⁴ Somnolence may have contributed to colonic relaxation after morphine, for sleep has previously been shown to induce colonic relaxation.¹ To counter this anticipated effect, subjects were encouraged to watch television and maintain a level of alertness. Opiate pathways are important at all levels of the colon, as the phasic contractions of the distal colon that occur postprandially can be inhibited by naloxone.¹⁵ In our experiments, morphine variably induced bursts of repetitive, phasic contractions in the sigmoid colon but it had less obvious effects in the descending colon.

Fasting and early postprandial phasic contractions were inhibited by atropine, as observed in earlier studies,¹⁶ but muscarinic blockade also dramatically reduced colonic muscle tone, making the colon much more compliant. Changes in segmental volume could have

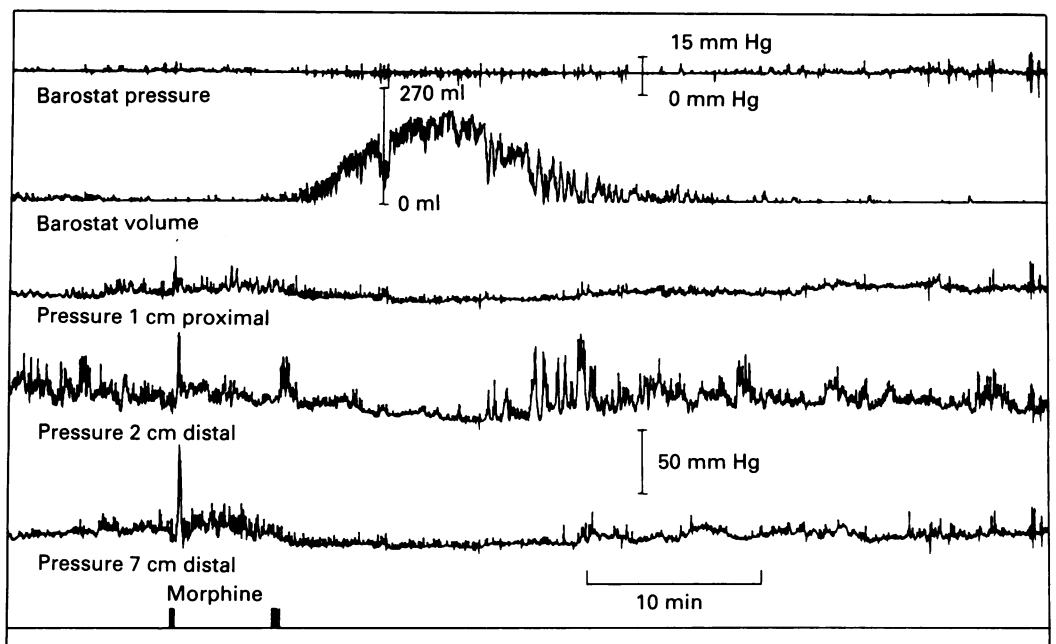


Figure 6: Barostat manometry recordings illustrating prompt, transient decrease in colonic tone after a slow (five minutes) intravenous injection of morphine that was given two hours postprandially when colonic tone was high (barostat balloon empty).

resulted from reduced haustral indentation of the lumen or from increases in the diameter of the segment being studied. By either means, the barostat balloon volume increased and, thus, the mean colonic diameter and segmental capacitance increased after atropine. Atropine did not inhibit the postprandial increase in colonic tone,¹⁸ though the response was slightly blunted. We interpret this to indicate that the postprandial increase in colonic tone is a non-cholinergic mechanism. One such mechanism could involve increased postprandial levels of cholecystokinin, stimulated by nutrients entering the duodenum^{17,18} 20–40 minutes after the meal.

Atropine also did not inhibit the generation of GMCs. We did not observe GMCs as a response to the inflated barostat balloon during fasting or in other studies of the postprandial period.¹ The atropine-meal sequence seemed, therefore, to be necessary. The barostat was able to demonstrate a period of prolonged hypertonicity that gradually reduced in magnitude after the passage of a GMC. Similar increases in tone follow the passage of GMCs down the cat colon, which was described as resembling 'a white bloodless rod.'¹⁹

Edrophonium, a short acting anticholinesterase, caused phasic contractile responses but did not change tone or cause GMCs. The muscarinic agonist, bethanecol, might have increased tone, as it did in the dog colon,⁸ and the lack of effect of edrophonium may have been related to its short duration of action.

Our experimental system did not seem to alter significantly the colon's responses to physiological stimuli nor to disrupt segmental, phasic contractions. In subjects who received placebo preceding the meal, the normal postprandial increase in phasic contractions was preserved.¹ In those experiments which featured both descending colon and sigmoid manometry, the normal proximal to distal gradient of activity was preserved, with the sigmoid colon usually being more active than the descending segment.

These experiments also show that modulation of colonic capacitance is not a phenomenon restricted to the proximal colon, where variations in the capacity of the colon to retain infused fluid have been shown using radionuclide techniques.⁴ The proximal colon with its saccular anatomy may be better suited to a reservoir role, but clearly the capacitance of the distal colon, even with its more tubular anatomy, is also subject to pharmacological control. Although changes in

capacitance may influence the transit of solids, tone is perhaps more relevant to the colonic transit of liquids or gas that are able to be 'squeezed' through the lumen. Thus, colonic relaxation may delay transit and promote the absorption of fluids and electrolytes, whereas increased colonic muscle tone may have the reverse effect and propel the contents of the colon towards the rectum.

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