Cholinergic giant migrating contractions in conscious mouse colon assessed by using a novel noninvasive solid-state manometry method: modulation by stressors

CURE/Digestive Diseases Research Center, and Center for the Neurobiology of Stress, Department of Medicine, Division of Digestive Diseases, University of California Los Angeles, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California

Submitted 16 July 2008; accepted in final form 17 March 2009

Gourcerol G, Wang L, Adelson DW, Larauche M, Taché Y, Million M. Cholinergic giant migrating contractions in conscious mouse colon assessed by using a novel noninvasive solid-state manometry method: modulation by stressors. Am J Physiol Gastrointest Liver Physiol 296: G992–G1002, 2009. First published March 19, 2009; doi:10.1152/ajpgi.90436.2008.—There is a glaring lack of knowledge on mouse colonic motility in vivo, primarily due to unavailability of adequate recording methods. Using a noninvasive miniature catheter pressure transducer inserted into the distal colon, we assessed changes in colonic motility in conscious mice induced by various acute or chronic stressors and determined the neurotransmitters mediating these changes. Mice exposed to restraint stress (RS) for 60 min displayed distal colonic phasic contractions including high-amplitude giant migrating contractions (GMCs), which had peak amplitudes >25 mmHg and occurred at a rate of 15–25 h−1 of which over 50% were aborally propagative. Responses during the first 20-min of RS were characterized by high-frequency and high-amplitude contractions that were correlated with defecation. RS-induced GMCs and fecal pellet output were blocked by atropine (0.5 mg/kg ip) or the corticotropin releasing factor (CRF) receptor antagonist astrressin-B (100 μg/kg ip). RS activated colonic myenteric neurons as shown by Fos immunoreactivity. In mice previously exposed to repeated RS (60 min/day, 14 days), or in transgenic mice that overexpress CRF, the duration of stimulation of phasic colonic contractions was significantly shorter (10 vs. 20 min). In contrast to RS, abdominal surgery abolished colonic contractions including GMCs. These findings provide the first evidence for the presence of frequent cholinergic-dependent GMCs in the distal colon of conscious mice and their modulation by acute and chronic stressors. Noninvasive colonic manometry opens new venues to investigate colonic motor function in genetically modified mice relevant to diseases that involve colonic motility alterations.

The mammalian colon plays important roles in the absorption, storage, and propulsion of luminal contents as part of the physiology of digestion (54). Appropriate motor activity is required to subserve these functions, as shown by altered colonic propulsive activity in several intestinal disorders including fecal incontinence, constipation, irritable bowel syndrome (IBS), and diverticular disease, as well as in pseudo-obstruction, diarrhea, inflammatory bowel disease (IBD), or diabetes (6, 9, 39, 57). However, the mechanisms underlying altered colonic motility and their contribution to symptom development are not well understood. These mechanisms are the subject of intensive clinical investigation using a variety of tools. The most common approaches employ endoscopic placement of side-hole manometry catheters and/or electrodes into a prepared colon (11, 23, 57). In experimental animals, colonic motility has been investigated typically by using EMG electrodes or strain gauges implanted in or on the colonic wall, respectively (14, 25, 41). Most other studies of colonic motor function in rats and particularly in mice have measured the effects of altered colonic motility, i.e., marker transit, bead expulsion, or defecation (2, 38, 46) as proxies of colonic motility. Although all methods used in experimental animals are highly valuable, either they are invasive and thus have inherent confounding factors due to surgery or, in transit assays, they do not provide adequate information on the spatiotemporal profile of colonic motility. The methodological constraints are more apparent in mice, where to date little is known about colonic motility in conscious state. Characterization of mouse colonic movement is mainly derived from either in vitro or isolated colon preparations (12, 33, 47, 48, 51). In particular, in the mouse isolated colon, the spontaneous motor activity comprises characteristic contractions, termed colonic migrating motor complex (CMMC) that are rhythmic, neurogenic, and propagative (12, 47, 58). However, the type, pattern, frequency, magnitude, and regulation of colonic motor activity in conscious mice are still not known. This contrasts with the vast knowledge of mouse colonic enteric neuron physiology (16, 44, 45, 53) and the availability of large and growing number of genetically modified mice with great potential as tools to help understand colonic motility and its impacts in health and disease states.

The colonic motility pattern of several species, in conscious state, is characterized by the appearance of phasic spike potentials and spontaneous high-amplitude migrating contractions termed long or short spike bursts (13, 15, 54) and giant migrating contractions (GMC) in conscious humans (55), dogs (21, 31), cats (22), and rats (25, 42). The form and pattern of colonic phasic contractions in these species vary depending on physiological status (feeding, fasting) and are altered by stress, colonic inflammation, and other diseases (4, 25, 56).

In the present study, we developed a novel noninvasive method to study distal colonic motility, using miniature catheter pressure transducer solid-state manometry, and characterized the patterns of distal colonic contractions in conscious mice. Since psychological stress either acute or chronic is known to alter colonic motility in experimental animals and humans (20, 61), we assessed the

Address for reprint requests and other correspondence: M. Million, CURE/Digestive Diseases Research Center and Center for Stress-Neurobiology, UCLA, VA Greater Los Angeles Healthcare System, CURE Bldg. 115, Rm. 118B, 11301 Wilshire Blvd., Los Angeles, CA 90073 (e-mail: mmulaget@ucla.edu).
distal colon contractile pattern in response to an acute restraint stress (RS) in naive or in chronically stressed mice, i.e., mice exposed to repeated RS or in corticotropin releasing factor (CRF)-overexpressing (OE) mice, a genetic model of chronic stress (8). Cholinergic and nitricergic pathways are known to be involved in the occurrence of rat colonic GMC (25), whereas both central and peripheral CRF receptors play role in RS-induced stimulation of colonic motility in rats (60). We therefore investigated the role of nitricergic, cholinergic, and CRF receptors in colonic contractility response to RS using pharmacological and neurofunctional approaches in conscious mice. The latter consisted of Fos immuno-histochemistry of the colonic longitudinal muscle myenteric plexus (LMPM) whole mount preparation as in our previous studies (63). We further validated the miniature pressure transducer solid-state manometry in conscious mice by assessing the time course of changes in the pattern of colonic contractions associated with abdominal surgery known to cause colonic ileus in mice (40).

MATERIAL AND METHODS

Animals

All experiments were conducted in nonfasted adult male mice (20–25 g, C57Bl/6; Harlan San Diego, CA) or male CRF-OE and wild-type (WT) mice (28–35 g) except otherwise stated. CRF-OE and their WT littersmates (Center for Neurobiology of Stress, Animal Models Core, University of California Los Angeles) were generated as previously described (59). The chimeric CRF transgene was composed of the rat genomic CRF gene replacing the 5′ regulatory region by a mouse metallothionein-1 promoter and microinjected into the male pronucleus of fertilized eggs (C57Bl/6 × SJL). Animals were housed (3–4/cage) and maintained under temperature- (20–24°C) and light- (12 h light-12 h dark) controlled environment. Animals were fed ad libitum with standard rodent chow (Prolab RMH 2500-5P14; Purina LabDiet, St. Louis, MO) and tap water. The experimental ad libitum with standard rodent chow (Prolab RMH 2500-5P14; Purina LabDiet, St. Louis, MO) and tap water. The experimental procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Substances and Treatments

Astrinex B (Peptide Biology Laboratory, The Salk Institute, La Jolla, CA) was synthesized by the solid-phase approach and the Boc strategy as previously described (50) and stored in powder form at −80°C. L-NAME (N^G-nitro-l-arginine methyl ester) and atropine sulfate (Sigma Chemical, St. Louis, MO) were kept in powder form at −4°C and room temperature, respectively. Immediately before use, astressin B was dissolved in distilled water and L-NAME and atropine were dissolved in saline (NaCl 0.9%). Intraperitoneal (ip) injections were made in a volume of 0.1 ml/mice.

Stress Models

Acute restraint. Restraint consisted of placing each mouse, for a 60-min period, in a Falcon plastic tube (2.7 cm diameter, 7–10 cm long, BD, Franklin Lakes, NJ) with several holes on the side and front for adequate ventilation (partial restraint tube device) as previously described (28, 35). The dimensions of the tube prevented the mice from turning around and limited their ability to move forward or backward.

Acute abdominal surgery. Under brief isoflurane anesthesia and aseptic condition, the mouse abdomen was incised, and the cecum was identified and palpated for 60 s with saline wet gauze as in our previous study (26). The control group (sham group) consisted of brief isoflurane anesthesia without performance of the surgical procedure.

Repeated restraint. Repeated RS consisted of performing the RS procedure described above for 1 h each day for 14 consecutive days.

Chronic CRF-OE mice. CRF-OE mice have been characterized as a genetic model of chronic stress in various behavioral, autonomic, and visceral responses (8).

Assessment of Colonic Motor Function

Intracolonial pressure recording procedure. Mice were briefly anesthetized with isoflurane (3% in O2), and a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX) lubricated with medical grade lubricant was introduced into the colon such that the middle of the pressure sensor (3.5 F) was 2 cm proximal to the anus. The catheter was then secured to the tail with tape, and colonic contractions were recorded in conscious mice immediately after their placement in the restraint tube. When required, two pressure transducer catheters were inserted at 2 and 4 cm proximal to the anus by the same procedure. Each pressure transducer catheter was connected to a preamplifier (model 600; Millar Instruments). The signal was then amplified by using a transducer amplifier in differential mode (TBM4, World Precision Instruments, Boca Raton, FL), acquired via a Micro1401 analog-to-digital interface (Cambridge Electronic Design) at 100 samples/s, and recorded with Spike 2 version 5 data acquisition software (Fig. 1, A and D). The system was calibrated by using known pressures...
at 0, 20, 40, and 60 mmHg at the start of each experiment to convert voltage output to intraluminal pressure. Abdominal contractions and breathing artifacts were excluded by smoothing the original trace with a time constant of 2 s (Fig. 1, B and E).

*Intracolonic pressure data analysis.* Colonic contractile pressure changes were quantified by measuring the area under the curve of the phasic component of the intraluminal pressure trace (pAUC) for every minute. The phasic component of intracolonic pressure was extracted from the original trace as previously reported (17, 18) by removing the direct current (DC) component with a time constant of 10 s from the 2-s smoothed original trace (Fig. 1, C and F). Since the main contractile colonic pressure occurred usually during the first 20 min, the mean pAUC was calculated for the time periods of 0–10, 10–20, and 20–60 min. Results are expressed as J) the time course of the phasic contraction, by calculating the mean pAUC for every minute (pAUCm) from the rolling average of pAUC for the period between 2 min before and 2 min after each minute’s pAUC (i.e., pAUCm = average of pAUC(m−2) min) or 2) the mean pAUC over a time period of 0–10, 10–20, and 20–60 min. In addition, frequency and amplitude as well as duration of contractions were determined for a total of 727 contractions obtained from 34 mice. The first 20 min of contractions were compared with the 20- to 60-min time period. In rats, the high-amplitude migrating distal colon contractions have pressure rise over 15 mmHg (10). Since in the present study we could not reliably attribute pressures below the 10 mmHg level to colonic contractions, we took the 10 mmHg as the detectable threshold and added the 15 mmHg rise, as defined by Croci et al. (10), and considered pressures over 25 mmHg as the threshold for defining an excursion in the pressure trace as a GMC. Although several studies identified and described CMMC as the main contractile pattern in isolated mouse colon (12, 47), there is so far only one study that reported colonic contractility in awake mice (40). In contrast, GMC has been described to be the main propulsive contraction in the colon of several species in conscious state, including in rats and humans (25, 55). Because the biochemical codes of GMCs described in conscious rat distal colon resemble that observed in the present conscious mice distal colon high-amplitude migrating contractions, we adopted the term GMC in the analysis and description of these contractions in the present study. 

*Propagation of GMCs.* In a separate study, propagation of GMCs was investigated in 14 mice by using two pressure probes placed at 2 and 4 cm proximal to the anus. The propagative nature of contractions >25 mmHg was evaluated by determining the order of appearance of the peak of the distal intraluminal pressure wave as described previously (47). Four different types of propagative and nonpropagative contractions were identified (Fig. 1, D–F). Contractions that propagate proximal to distal in sequence from the 4-cm to the 2-cm site were classified as aborally propagating contractions whereas contractions that propagate distal to proximal from the 2-cm to the 4-cm site were classified as orally propagating contractions. These propagative contractions usually display similar shapes although their amplitude may vary. Nonpropagative contractions are those that appear only at the 4- or 2-cm site. Lastly, contractions that appear simultaneously at both the 4- and 2-cm sites are considered as other types of contractions. The aborally propagated contractions were considered as propagated when detected at the level of 2 cm during an interval between 2 and 120 s after detection at 4 cm. The time of occurrence of such a contraction was set at the peak of its detection at the 4-cm site. The speed of propagation in mm/s was obtained by dividing the distance between the two pressure sensors (20 mm) by the time (s) elapsed between the peaks of the contractions at 4- and 2-cm sites. Of the 14 mice, 6 did not expel a fecal pellet during the 1-h recording period and displayed a subcircular-like motility pattern, probably because of the presence of the two pressure probes. Thus the two probes study was only used to investigate propagative properties of GMCs that were analyzed on a total of 165 contractions from 8 recordings (1/mouse).

**Fecal pellet output.** In nonfasted conscious mice, defeation was monitored as described previously by counting the number of fecal pellets excreted for 1 h (35), along with colonic contractions recording. Correlation with colonic motility was determined by comparing the number of fecal pellets output to the normalized first 20-min colonic motility. Normalized colonic motility was calculated by dividing the mean pAUC of the first 20 min by the mean pAUC of the last 40 min. This provided a representative value of the initial activation of colonic motility in response to acute stress and thus allows intraindividual comparison among mice. The reasons for making a correlation study between the 60-min fecal pellet output and the first 20 min of normalized colonic contractions are that 1) because the presence of catheters in the colon in the RS conscious mice, fecal pellet output can only be measured at the end of the stress session (60 min); 2) several studies have shown that more than 80% of fecal pellet output induced by RS in rats and mice occurred in the first 30 min of the stress session (28, 34, 35); and 3) the present data show that the robust colonic contractile response to RS occurs in the first 20-min period, in agreement with the second point above. Thus to validate the use of miniature pressure transducer solid-state manometry to monitor propulsive colonic motility, we assessed whether there was a correlation between the first 20 min of colonic contractions with the classic colonic propulsive assay, the 60-min fecal pellet output.

**Fos Immunohistochemistry**

Mice were euthanized by cervical dislocation, and the abdominal cavity was opened. The distal colon was dissected out and opened longitudinally along the mesenteric border, and longitudinal muscle myenteric preparation was prepared and processed for Fos immunohistochemistry as detailed previously (63). The presence of Fos immunoreactivity (IR) was revealed as a dark brown precipitate located in neuronal nuclei in a microscope (Zeiss, Axiolab II). Fos-IR cells were counted in 20 ganglia of each mouse LMP preparation from the distal colon.

**Experimental Protocols**

**Effect of RS on colonic motility in conscious mice.** ACUTE RS IN NAIVE MICE PRETREATED OR NOT WITH CRF ANTAGONIST. Immediately after transducer placement, naive mice were injected ip with saline (n = 12) or astressin B (100 μg/kg, n = 6) and 5 min later were placed in the restraint tube and left undisturbed whereas colonic contractions were recorded for a 1-h period. Dose of astressin B was based on our previous studies (29).

ACUTE RS IN MICE EXPOSED TO REPEATED RS. Mice (n = 6) were exposed to daily RS (1 h/day × 14 days) were placed on day 15 in the restraint tube immediately after transducer placement, and colonic contractions were monitored as described above for 1 h.

ACUTE RS IN CRF-OE MICE. CRF-OE (n = 10) and WT (n = 4) were placed in the restraint tube immediately after transducer placement, and colonic contractions were monitored as described above for 1 h. Because WT littermate and the C57Bl/6 WT mice (n = 6) had similar contractions patterns, data were pooled together and used for the WT group.

**Effects of Cholinergic and Nitrergic Blockade on Acute RS.** Mice were injected ip with saline (0.1 ml), atropine (0.5 mg/kg), or L-NAME (10 mg/kg) during anesthesia, 3–5 min before being placed in the restraint tube, and colonic contractions were recorded for 60 min after mice placement in the tube. Doses of atropine and L-NAME were adopted from our previous studies in rats (27).

**Effects of acute RS on Fos expression in the mouse distal colonic myenteric plexus.** Mice were placed in a restraint tube or kept in their home cage and were euthanized 60 min later. Distal colons were then collected for immunohistochemical staining of Fos in the LMP neurons.

**Effects of abdominal surgery.** Conscious mice were first placed in the partial restraint stress device and baseline colonic contraction was monitored for 60 min. A group of mice (n = 6) were brieﬂy anesthetized with isoflurane (3% in O2) and a laparotomy plus a 60-s...
cecal palpation was performed. The sham surgery group (n = 6) underwent only a brief anesthesia without laparotomy and cecal palpation. Mice were then put back into the restraint device and colonic contraction was recorded for another 60 min.

Data Analysis

Values are presented as means ± SE. Influence of treatment was calculated by ANOVA followed by a Student-Newman-Keuls post hoc test, and the time course effect in each group was analyzed by ANOVA for repeated measures followed by a Student-Newman-Keuls post hoc test whereas interindividual pairwise comparisons (baseline vs. response) were performed with a nonparametric Wilcoxon’s test whereas interindividual comparisons (control vs. treated) were investigated by running a nonparametric Mann-Whitney test. Correlation between colonic motility and fecal pellet output was done by Spearman rank-order correlation. Mean values of Fos IR in the distal colon LMMP were compared by Student’s t-test. A P value <0.05 was considered significant.

RESULTS

Characterization of Colonic Motility and GMCs in Acutely Stressed Conscious Mice

Mice placed in the restraint tube displayed mainly low- (≤25 mmHg) and high-amplitude (>25 mmHg) colonic contractions. Further analysis showed that the first 20 min, after mice were placed in the restraint tube, were marked by a robust transient activation of colonic contractions (Fig. 2, A–C). The mean pAUC for the 0- to 20-min time period was significantly increased compared with the subsequent 20- to 60-min period (P < 0.05; Fig. 2C). The overall transient increase in colonic contractions was associated with an increase in frequency of >10 mmHg contractions compared with the 20- to 60-min time period (55.8 ± 6.6 vs. 31.1 ± 2.7 contractions/h, respectively; P < 0.0001). In addition, during the first 20 min, there was a significant increase in low-amplitude (≤25 mmHg) (P < 0.01) (Fig. 3, A and B) and short-duration (<20 s) (P < 0.01) (Fig. 3, A and B) contractions compared with the 20- to 60-min period. Recordings from the mouse distal colon showed high-amplitude (>25 mmHg) contractions, referred as GMC (Fig. 1; Figs. 2A and 3A) similar to that reported in other species (10, 25). The frequency of GMC as well was increased during the first 20 min compared with those in the 20- to 60-min period (25.2 ± 3.3 vs. 16.8 ± 2.6 contractions/h; P < 0.05). GMCs represented 54.3% and 45.3% of the total contractions of the 0- to 20-min and 20- to 60-min time periods, respectively. Phasic contractions with amplitude less than 10 mmHg were often not detectable. When detectable, their mean frequency was 4.8 ± 0.3 contractions/min with a range of 3.6–6.0 contractions/min.

Study on propagation of GMCs showed that the overall frequency of high-amplitude contractions (>25 mmHg) detected at the 4- and 2-cm site was 20.5 ± 1.5 and 20.6 ± 3.2 h⁻¹, respectively. Of these the number of contractions that propagated aborally from 4-cm site and identified at the 2-cm site had a frequency of 10.6 ± 1.9 h⁻¹, representing 51.8% of the GMCs (Fig. 1). The propagated GMCs were of higher amplitude compared with the nonpropagated GMCs (60.9 ± 2.9 vs. 47.6 ± 2.3 mmHg, respectively; P < 0.001; Fig. 4, A–C). The colonic phasic contraction pressure at which 50% of the contractions would propagate aborally was 30 mmHg (Fig. 4, A and B). In addition, the likelihood for a contraction to be propagated aborally was positively correlated with its amplitude (P < 0.001) yielding 59.7% and 92.3% propagation rate.
for contractions over 30 and 90 mmHg, respectively (Fig. 4, A and B). The mean velocity of aborally and orally propagated GMCs were 1.0 ± 0.1 and 1.1 ± 0.2 mm/s, respectively and were not correlated with the amplitude of the GMCs (P = 0.60). The orally propagating GMCs had a frequency of 1.0 ± 0.3 h⁻¹ whereas the number of contractions that did not propagate to the 2-cm site and those contractions that occurred at both sites simultaneously were 7.8 ± 1.6 and 1.1 ± 0.4 h⁻¹, respectively.

**CRF Receptor Blockade Prevents Acute RS-Induced Distal Colonic Contractions and Fecal Pellet Output**

In saline-pretreated mice, in the first 20 min colonic contractile response was higher than in the subsequent 40 min (Fig. 6, A–C). The overall 60 min pAUC was 222 ± 24 mmHg × min. This was decreased to 81 ± 10 mmHg × min in mice pretreated with atropine (0.5 mg/kg ip). Similarly atropine, compared with saline, reduced the 60-min pellet output (11 ± 0.4 vs. 4 ± 1.4 pellets/60 min; P < 0.05). However, pretreatment with L-NAME (10 mg/kg ip) only decreased significantly the pAUC for the first 0–10 min period (Fig. 6, A–C) and had no significant effect on the 60 min AUC (202 ± 17 mmHg × min) or fecal pellet output (8.5 ± 1.3 h⁻¹).

**Atropine Blocks Acute RS-Induced Distal Colonic Contractions and Fecal Pellet Output**

Pretreatment with astressin-B (100 μg/kg ip in 0.1 ml), a CRF receptor subtype 1 (CRF₁) and CRF receptor subtype 2 (CRF₂) antagonist, prevented the transient increase in colonic contractions compared with the saline (0.1 ml ip)-treated group (Fig. 5, A–C) as evidenced by the significant decrease in mean pAUC for the 0–10 min and 10–20 min time periods (Fig. 5C). This was mainly due to the decrease in the frequency of all contractions (55.7 ± 6.6 vs. 35.0 ± 4.1 contractions/h; P < 0.05), and more specifically of the short-duration (<20 s) contractions (41.5 ± 4.6 vs. 21.0 ± 3.6 contractions/h; P < 0.05) (Fig. 5D) and of GMCs (8.4 ± 1.1 vs. 3.8 ± 0.7 contractions/h; P < 0.05). Similarly, astressin B attenuated the fecal pellet output induced by RS (9.5 ± 1.6 vs. saline 13.7 ± 1.4 pellets/h; P < 0.05).
Acute RS Induces Fos Expression in Distal Colonic Myenteric Ganglia

Distal colon LMMP from control mice showed no or few Fos-expressing neurons (0.36 ± 0.17 cells/ganglion, n = 4, Fig. 7, A and B). However, acute RS (60 min) induced Fos-IR in distal colonic myenteric ganglia neurons (3.63 ± 1.35 cells/ganglion, n = 6) (Fig. 7, A and B). The presence of Fos-IR was restricted to cells located within the myenteric ganglia, and no Fos-IR was observed in smooth muscle cells (Fig. 7A).

Acute and Chronic Stresses Differentially Affect Colonic Motility in Conscious Mice

Mice exposed to an acute RS, 24 h after chronic intermittent RS (1 h/day for 14 days), had a robust immediate stimulation of phasic colonic contraction that was shorter in duration (first 10 min) than the initial stimulation observed in naive mice that were subjected to a single RS (first 20 min) (Fig. 8, A–C). The relatively short-lasting activation of the colon in the chronically stressed mice was associated with a significant decrease in the number of short-duration (<20 s) contractions but not of GMCs (>25 mmHg amplitude) (Fig. 8D). In the genetic model of chronic stress, CRF-OE mice exposed to a single RS exhibited a colonic motor pattern similar to that of mice subjected to chronic intermittent RS. In CRF-OE mice, the stimulated colonic response lasted only for the first 10 min (Fig. 8, A–C) and was associated with a decrease in the frequency of short-duration contractions, but not that of GMCs (Fig. 8D). Fecal pellet expulsion was similar in chronic RS mice or in CRF-OE mice compared with controls under acute RS [12.3 ± 1.0 and 9.8 ± 1.9 pellets/h, respectively; P > 0.05 compared with acute RS (13.7 ± 1.4 pellets/h)]. When detectable, phasic contractions below 10 mmHg had a similar frequency in both chronic RS and CRF-OE mice (5.0 ± 0.4 and 6.2 ± 0.4 contractions/min, respectively; P > 0.05 compared with acute RS).

Association of Colonic Contraction and Fecal Pellet Output

Previous studies have shown that over 80% of the fecal pellet output response to acute stress occurs in the first 30 min of the stress sessions (28, 34, 35). In the present study setting, colonic contractile response was continuously recorded for 60 min. In acutely RS-nontreated mice the initial 20-min colonic contractile response and the overall 60-min fecal pellet output were significantly higher than in the atropine- or astressin B-treated mice (see Figs. 5 and 6). However, because of the presence of catheter in the colon, fecal pellet output could only be counted at the end of the 60-min RS. Data from a total of 60 mice showed that the fecal pellet expulsion and normalized first 20 min distal colonic contractions were significantly correlated (r = 0.43, P < 0.05) (Fig. 9).

Postoperative Ileus Reduces Colonic Contraction in Mice

Colonic contractions recorded for the 30-min baseline in both groups were comparable (mean pAUC for 30 min baseline: 142 ± 30 and 129 ± 34 mmHg × min for sham and postoperative ileus groups, respectively; P > 0.05; Fig. 10, A–C). Sham procedure (anesthesia only) tends to decrease the 1-h mean pAUC, but this effect was not significant compared with baseline (114 ± 18 mmHg × min; P > 0.05; Fig. 10, A–C). By contrast, a marked reduction of the 1-h mean pAUC...
The present study, using a novel noninvasive miniature pressure transducer manometry, characterized the pattern, frequency, amplitude, and speed of propagation of distal colonic contractions in conscious mice under various experimental conditions. The distal colonic contractions of naive mice exposed to acute RS is characterized by the apparition of low-amplitude (<25 mmHg) phasic contractions and high-amplitude (>25 mmHg) GMC that is correlated with fecal pellet output. These contractions are blocked by atropine but not by L-NAME, suggesting a primary role of cholinergic pathway in distal colon motor activity in conscious mice. Acute RS-induced stimulation of colonic motor activity in conscious mice was also prevented by the CRF receptor antagonist astressin B injected peripherally, indicating the role of CRF receptors in the response. In chronically stressed mice, either in a genetic model of CRF-OE mice or in mice exposed to daily 1-h RS for 14 days, the colonic phasic motor activity to RS, compared with control mice, is characterized by a shorter duration of the initial increase in colonic phasic contractions. The noninvasive miniature pressure transducer manometry allows us to detect the hitherto unidentified alterations of colonic contractions and/or motility patterns in response to various treatments in conscious mice.

Distal Colonic Motor Profile in Conscious Mice

Using the novel noninvasive manometry method, we showed that, in conscious mice placed in the restraint tube, distal colon exhibits primarily low-amplitude (<25 mmHg) phasic and high-amplitude (>25 mmHg) contractions throughout the recording period. The high-amplitude contractions in the distal colon constitute over 50% of the contractions and occur at a frequency of 15–25 h⁻¹, comparable to that described in the isolated mouse colon (one contraction every 2–5 min) (12, 47) and the distal colon of conscious rats (17 h⁻¹) (25). However, they are much higher than the distal colonic GMCs reported in dogs (3 h⁻¹) (21) and the 1–2 day⁻¹ high-amplitude propagative contractions in human colon. Although only half of the overall mice distal colon GMCs propagates aborally, the proportion of such contractions that propagate distally increases as the contraction pressure rises. Most of the contractions with >75 mmHg propagate distally at a speed of about 1.0 ± 0.1 mm/s. The relatively higher number of GMCs in mice compared with large animals including humans is likely to be related to the type of stool as well as the increased frequency and number of fecal pellets expelled in mice. The higher number of propagative GMCs in mice (reaching over 50% of the 15–25 contractions/h), than in rats (<5 h⁻¹) (10, 25) could also be related to the increased number of fecal pellets expelled in mice than rats per unit of time. However, the presence of two probes may have contributed to the increased rate of propaga-
contractions that is composed of three, and sometimes four, distinct phases that are defined in terms of the amount and regularity of contractile or electrical spiking activity. In our in vivo study, colonic contractions appeared in a random manner with no apparent MMC pattern. Four, the term GMC has been used for physiologically comparable colonic contractions in several species including conscious rat (25, 42), dogs (21, 31), cats (22), and humans (55). Given that the in vitro colonic tissue is devoid of extrinsic innervation and lacks the natural milieu of abdominal cavity pressure, difference between the in vitro and the conscious mouse colon motility pattern is not surprising. Similar differences have also been reported between in vivo vs. in vitro rat colon motor pattern and neuromediators roles on colonic motility (25). Further studies are needed to dissect out the chemical codes and neuronal pathway similarities and differences of colonic contractions in mice in vitro and in vivo (awake and anesthetized) to accurately characterize the mouse colon contractions.

**Validity of Solid-State Manometry for Conscious Mouse colon Contraction Measurement**

Appropriate methods to measure colonic motility pattern should be able to detect colonic motor events comparable to or better than the existing methods and be able to detect effects of inhibitory or excitatory stimuli known to modulate colonic motor function. The simultaneous measurement of colonic motor response by a classical method of fecal pellet output and the present manometry based colonic contractions show a positive correlation between the two methods. Similarly, the robust initial (first 20 min) colonic contractions detected by manometry in the present study is in line with the time course of fecal pellet output in rodents where over 85–90% of fecal expulsion in response to acute stressors occurs during the first 15 min (34). Contractions after the first 20 min are decreased to low levels with the overall colonic activity remaining the same for 40 min and even beyond the 60 min in few of the recordings made (data not shown). Another condition known to influence colonic motility is abdominal surgery that induces a postoperative ileus in humans and experimental animals including in mice (40). Postoperative colonic ileus in mice has been studied primarily by using gastrointestinal transit assays (43) and in one study by recording intracolonic pressure in awake restrained mice (40). We provide here evidence that both low-amplitude phasic contractions and high-amplitude GMCs are inhibited following abdominal surgery and 1-min cecal palpation in mice. This is in agreement with the reduction of large pressure waves of short duration, reported in awake mice (40). The above points argue in favor of a strong face validity of colonic contractions measured by the present miniature pressure transducer manometry in response to various stimuli known to influence colonic transit in conscious mice.

In addition, the present data show that colonic contractions including GMCs are atropine sensitive, suggesting a role of muscarinic pathway in the occurrence of colonic GMCs in conscious mice. Cholinergic involvement in the distal colon motor activity has also been shown in rats (25). However, L-NAME had no effect on the overall 60-min distal colonic contractility and only mildly attenuated the initial 10-min response. Given that NO is one of the inhibitory neurotrans-

---

**Fig. 10. Effect of interoceptive stress, abdominal surgery, on distal colonic contractions in conscious mice.** Under brief isoflurane anesthesia mice underwent either sham treatment (only anesthesia) or abdominal surgery (abdominal incision, opening of the peritoneum, and palpation of the cecum for 1 min) and suturing of the incision. Colonic contractions before the surgery (30 min) and after surgery (60 min) were recorded and compared. A: representative raw trace of distal colonic contractions in sham (top) and surgery mice (bottom). B: time course of the baseline and postsurgery period AUC in sham and surgery groups. C: total baseline and postsurgery AUC in sham and surgery groups. *P < 0.05 vs. baseline and sham; n = 6/group; ANOVA.
mitters in the gastrointestinal tract (5, 30), L-NAME was expected to cause contractions. The lack of stimulatory effects of L-NAME on conscious mouse distal colonic contraction may relate to 1) RS-induced mouse colonic contractions may primarily involve excitatory neurotransmitters such as acetylcholine, as shown by the blocking effect of atropine, whose effect is not affected by L-NAME, and 2) NO in the distal colon may trigger cell signaling that does not involve cyclic GMP, such as extracellular Ca^{2+} influx through L-type Ca^{2+} channels that could result in contraction rather than relaxation (24). The latter could also explain the moderate inhibitory effect of L-NAME seen in the first 10 min of the colonic response. Several studies have also shown a region specific lack of L-NAME stimulatory effect in the gastrointestinal tract (25), particularly when the colon is under a stimulated state (19). It is nevertheless possible for NO to be involved in the propagation of the high-amplitude waves and transit by facilitating descending relaxation as has previously been described in rats (37). The present data on L-NAME provide also a basis to further study whether and how NO exerts different effects on basal vs. stress-induced colonic contractions.

In rodents, it has been shown that acute stress-induced stimulation of colonic motor function involves activation of central and peripheral CRF signaling in both rats and mice (60). The present study shows that increased distal colonic contractions are blocked by the peripheral administration of CRF₁ and CRF₂ receptor antagonist astressin B, in agreement with evidence that peripheral CRF receptor play a role in colonic response to exteroceptive stress (62, 64). Taken together, the successful use of still manometry to characterize colonic contractions in conscious mice placed in restraint tubes using astressin B as well as atropine and L-NAME lends support to the construct validity of the still manometry to measure conscious mouse colonic contractions reliably. We also showed that acute RS activates colonic myenteric neurons, as evidenced by Fos expression. It is likely that the activated enteric neurons represent a population of cholinergic motor neurons that may be activated through CRF pathways. We reported in the rat colon that intraperitoneal CRF peptide administration activates cholinergic myenteric neurons through CRF₁-dependent receptors located on myenteric neurons (32, 64). Of significance is also that the colonic contractile response to restraint stress is characterized by an initial higher response (the first 20 min) followed by a lower and stable activity (20- to 60-min recording period). Astressin B selectively blocked the initial robust response to 60-min recording period). Astressin B selectively blocked the initial robust response to stress, without affecting the subsequent basal like response, as well documented (28, 35). The evidence that astressin does not have effect on basal colon motor activity (28, 34–36), coupled with the present data in which astressin B blocked the initial robust response without affecting the subsequent 20- to 60-min period response, suggests that the later non-astressin B-sensitive low and stable contractile pattern may represent a basal type of activity. A definitive description of conscious mice distal colon baseline contraction pattern, however, requires recordings from freely moving mice, in the presence or not of a catheter, a method that is not yet available.

### Chronic Stress and Colonic Motor Pattern in Conscious Mice

Although chronic stress is the most relevant event in stress-related gut function alterations, to date the influence of chronic stressors on colonic motility in conscious mice as well as its underlying pathways have never been investigated. In addition, the few existing reports on chronic stress-related colonic fecal pellet output in rats are inconsistent in that an increase (52), decrease (1), or absence of effect (35) have been reported. Such variability is likely to be due to the use of different stress protocols and strains of animals along with the fact that fecal pellet output does not provide spatiotemporal information of motor events occurring in the colon. In the present study, fecal pellet output response was similar in the acute stress as well as the chronic stress groups, i.e., chronic repeated RS or in CRF-OE mice. However, we found a significant decrease in the duration of the initial colonic contraction in chronically stressed mice (10 min) compared with the acute stress controls (20 min). Similar pellet output between mice pretreated with L-NAME or saline and submitted to acute RS was also observed despite the short initial colonic response in the L-NAME-pretreated mice, suggesting that initial stimulation as short as 10 min is enough to cause maximal pellet output response to RS. Therefore, solid-phase colonic manometry method provides additional information on the motility pattern that could not been detected in the classical transit measurement methods and could be of great value to provide a physiological basis for chronic stress-related symptoms of colonic motor function alterations. Although CRF pathway was not explored in the chronically stressed mice, the striking similarity between the colonic contraction patterns of the chronic partial restraint stress and the CRF-OE mice with regards to the time and space organization of contractions suggests that such modifications may be related to CRF pathway. For instance, CRF₂ signaling has been shown to play a stress-coping role to attenuate the magnitude and duration of the endocrine and defecation responses to stress in mice (7, 35, 49). Whether such a mechanism develops in chronically stressed mice is not known and requires further studies.

In summary, conscious mice distal colonic contraction involves rhythmic apparition of high- and low-amplitude phasic contractions. Mice display a higher number of high-amplitude propagative contractions than that reported in other mammalian species, consistent with the elevated number of fecal pellets expelled per unit time in mice. The study provides also evidence that acute RS-induced distal colonic contractility in conscious mice involves cholinergic and CRF signaling pathways and activates colonic myenteric neurons. Chronic stress does not affect the fecal pellet output response to an acute RS in mice, but leads to different colonic contractility pattern. Solid-state colonic manometry is a noninvasive and reliable method to monitor colonic motility in conscious mice and opens a new venue to measure subtle and discrete colonic motility changes. It is of note however, that whereas the new tool is easily and reliably used for a single site recording, simultaneous recording form several colonic regions without compromising the potency of the colon for luminal flow necessitates incorporation of multiple miniature transducers on a single probe. In view of the availability of several genetically modified mice and absence of tools to assess colonic motility pattern

AJP-Gastrointest Liver Physiol • VOL 296 • MAY 2009 • www.ajpgi.org
in awake mice, solid-state manometry will offer a valuable alternative to study the hitherto unknown awake mice’s normal and altered colonic motor activity and is poised to help better understand the pathophysiological basis for stress-related gut dysfunction.

ACKNOWLEDGMENTS

We thank Dr. J. Rivier (The Salk Institute, Peptide Biology Laboratories) for the synthesis and gift of astressin-B, Dr. M. Fanselow (R24AT00268) for the CRF-OE mice, and Honghui Liang for skillful technical assistance.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants R21 DK-068155 and RO1 DK078676 (M. Million), DK-57238; Veterans Affairs Career Scientist Award and Veterans Affairs Merit Award (Y. Taché), the French Foreign Office (Egidie program) and the French Society of Gastroenterology (S.N.F.G.E.) (G. Gourcoul).

REFERENCES


COLORECTAL MANOMETRY IN CONSCIOUS MICE


64. Yuan PQ, Million M, Wu SV, Rivier J, Taché Y. Peripheral corticotropin releasing factor (CRF) and a novel CRF1 receptor agonist, stressin-1A activate CRF1 receptor expressing cholinergic and nitricergic myenteric neurons selectively in the colon of conscious rats. *Neurogastroenterol Motil* 19: 923–936, 2007.