Am J Physiol Gastrointest Liver Physiol 286: G321–G332, 2004;
10.1152/ajpgi.00057.2003.

Coordinated gastric and sphincter motility evoked by intravenous CCK-8 as monitored by ultrasonomicrometry in rats

David W. Adelson, Mulugeta Million, Koki Kanamoto, Tiffany Palanca, and Yvette Taché

Center for Ulcer Research and Education: Digestive Diseases Research Center and Center for Neurovisceral Sciences and Women's Health, Division of Digestive Diseases, David Geffen School of Medicine, University of California, and Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California 90073

Submitted 6 February 2003; accepted in final form 12 August 2003

Adelson, David W., Mulugeta Million, Koki Kanamoto, Tiffany Palanca, and Yvette Taché. Coordinated gastric and sphincter motility evoked by intravenous CCK-8 as monitored by ultrasonomicrometry in rats. *Am J Physiol Gastrointest Liver Physiol* 286: G321–G332, 2004; 10.1152/ajpgi.00057.2003.—Gastric and sphincter motility evoked by intravenous injection of CCK-8 were investigated in urethane-anesthetized rats. Digital ultrasonomicrometry was used to monitor pyloric (PYL), antral (ANT), corpus (COR), and lower esophageal sphincter (LES) movements while simultaneously measuring intragastric pressure (IGP) and, in some experiments, subdiaphragmatic intraesophageal pressure (sIEP). Intracrystal distances (ICD) were measured continuously between pairs of piezoelectric crystals affixed to the serosa of PYL, ANT, COR (circular and longitudinal), and LES. Consecutive intravenous injections of CCK-8 (0.3, 1, and 3 μg/kg) at 30-min intervals caused dose-dependent simultaneous tonic contractions of PYL and ANT, LES opening, and drops in IGP with peak changes at 3 μg/kg of −17.9 ± 2.1, −7.7 ± 2.5, 6.5 ± 1.4, and −29.2 ± 3.8%, respectively, whereas intravenous saline had no effect. Rhythmic contractile activity was inhibited by CCK-8. COR responses were not significantly different from vehicle controls for most metrics, and the direction of response for circular COR varied between preparations, although not for repeated trials in a single preparation. During the LES response to CCK-8, sIEP rose in parallel with drops in IGP, indicating formation of a common cavity. Recovery of LES ICD after intravenous CCK occurred more rapidly than recovery of PYL ICD, suggesting the importance of preventing simultaneous patency of gastroesophageal and gastroduodenal passages. The CCK_A receptor antagonist devazepide (500 μg/kg intravenous) inhibited motion responses evoked by intravenous CCK-8. These data revealed CCK-8-induced gastric and sphincter activity consistent with retropulsion of gastric content.

lower esophageal sphincter; pylorus; gastric intraluminal pressure; antrum; devazepide

CCK THAT IS RELEASED FROM mucosal cells of the proximal duodenum in response to the presence of lipid alters gastric motility, inhibits gastric emptying, and produces sensations of satiety (9, 10, 21, 27). Due to the importance of CCK in the regulation of gastrointestinal (GI) motor function and feeding behavior, a large number of studies have examined the motility effects of CCK on the whole stomach or on specific regions [antropyloric, fundus, and lower esophageal sphincter (LES)] along with the pathways that mediate these effects. Intravenous injection of active fragments of CCK has been shown to reduce intragastric pressure (IGP) in duodenally ligated rats (20), to contract the pylorus (PYL; 12, 17, 18), and to relax the LES in cats (2) and humans (13) but not in opossums (6). Additionally, CCK is believed to contribute to the forestomach accommodation accompanying ingestion of a lipid-rich meal in experimental animals and humans (15, 19, 26).

To date, temporal relationships between CCK-induced motility changes in different regions of the stomach have not been described in detail, possibly because, in part, of methodological constraints. In the present study, we applied digital ultrasonomicrometry as a novel technology to study GI motility. Although sonomicrometry has been used in the field of cardiovascular physiology since the mid-1950s (22), its applicability to the study of GI motility has been little explored.

The objectives of the present study were: *1*) to use digital ultrasonomicrometry to investigate the coordinated movement responses in PYL, antrum (ANT), corpus (COR) (circular and longitudinal), and LES induced by increasing doses of CCK-8 injected intravenously in urethane-anesthetized rats; *2*) to simultaneously monitor movement responses and IGP changes and investigate the relationship between them; and *3*) to determine the role of the CCK_A receptor in mediating motional responses to intravenous CCK-8, using the selective CCK_A receptor antagonist devazepide (5).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (270–310 g; Harlan Laboratories, San Diego, CA) were housed under controlled conditions of temperature (22–24°C) and illumination (12:12-h light-dark cycle, starting at 6:00 AM) and maintained with Purina Chow and tap water ad libitum. All experiments were begun between 9:00 and 11:00 AM in rats deprived of food overnight (>14 h) but allowed free access to tap water up to the beginning of the experiments. Rats were anesthetized by injection of 25% urethane (1.5 ml/kg ip), followed by additional doses of ≤0.2 ml ip 25% urethane, if needed, to render the animal areflexic to strong ear or tail pinch before surgery was initiated. Protocols were approved by the Veterans Administration Institutional Animal Care and Use Committee (ACORP 96–080–08).

Respiratory and Body Temperature Monitoring

Animals were tracheally cannulated with a Y-tube cannula to allow free breathing through one branch while monitoring respiratory rate and pattern (RESP) via a heated pneumotach (Hans Rudolph, Kansas City, MO) attached to the output of the other branch. Rectal temperature was monitored continuously via a thermistor probe and thermometer with analog output (YSI, Yellow Springs, OH). An intravenous jugular cannula was inserted to allow continuous infusion of 0.4 ml/h sterile saline to maintain hydration and for intravenous injection of test substances.

Address for reprint requests and other correspondence: D. W. Adelson, CURE: Digestive Diseases Res. Ctr., VAGLAHS–West LA VAMC, 11301 Wilshire Blvd., Los Angeles, CA 90073 (E-mail: dadelson@ucla.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

Gastric Luminal Pressure Monitoring

Laparotomy was performed, and a small opening was also made in the abdominal flank. The duodenum was ligated at least 1 cm distal to the PYL and proximal to the bile duct. A small incision was made in the nonglandular forestomach, and any residual gastric contents were flushed out onto a gauze pad. To allow intragastric injection or withdrawal of saline, a silicone rubber cannula (3 mm outer diameter; 1 mm inner diameter) was passed through the flank hole and inserted via the gastric incision into the COR. The tip of the cannula, which had large side holes to prevent clogging, was positioned at the distal end of the COR, and the gastric incision was closed around the cannula by using silk sutures. A catheter pressure transducer (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX) extending to the tip of the gastric cannula was used to record IGP.

Catheter pressure transducers were connected to a preamplifier (model 600; Millar Instruments, Houston, TX), the output of which was subsequently amplified via a single-ended connection to the transducer amplifier.

Ultrasonometric Measurements of Gastric Motility

Piezoelectric crystals 1 mm in diameter (Sonometrics, London, ON, Canada) were affixed to the serosal surface of the stomach using a small drop of cyanoacrylate glue (Vetbond; 3M Animal Care, St. Paul, MN). Crystals were placed as pairs 2–6 mm apart and oriented parallel to the circular muscle in PYL, ANT, circular COR (CORci), and LES (Fig. 1). LES crystals were placed along the line of the esophagocardiac junction. CORci crystals were placed several millimeters aborad to the dividing line between the glandular and nonglandular portions of the stomach, and ANT crystals were placed 0.5–1 cm orad to the PYL. PYL crystals were placed at the antroduodenal junction at its narrowest point. In several experiments, instead of measuring the response of ANT, an additional crystal was placed in the COR so as to allow recording of the longitudinal (i.e., parallel to the rugae) motion of the longitudinal COR (CORln). In this arrangement, one of the three COR crystals was a member of both the CORci and the CORln crystal pairs. After the crystals were placed, the abdominal cavity and skin flaps were reclosed using silk sutures and cyanoacrylate glue.

Data Acquisition and Analysis

Sonometric distance measurement data were acquired digitally at 50 samples/s via a digital sonomicrometer (model TRX-13; Sonometrics) connected to a Pentium III class computer running SonoLAB software (Sonometrics). The level at which the arrival of the ultrasound wavefront was recognized (triggering) was set by observing the arriving wavefront on an oscilloscope and adjusting the triggering level so that the first peak in the wavefront was reliably marked. Digitally acquired distance data were simultaneously output as analog signals via an installed four-channel digital-to-analog converter. These sonometric analog signals along with all analog physiological data (rectal temperature, RESP, and IGP) were acquired using a Micro1401 A/D interface (Cambridge Electronic Design) connected to a Pentium II class computer running Spike 2 (Cambridge Electronic Design) data acquisition software.

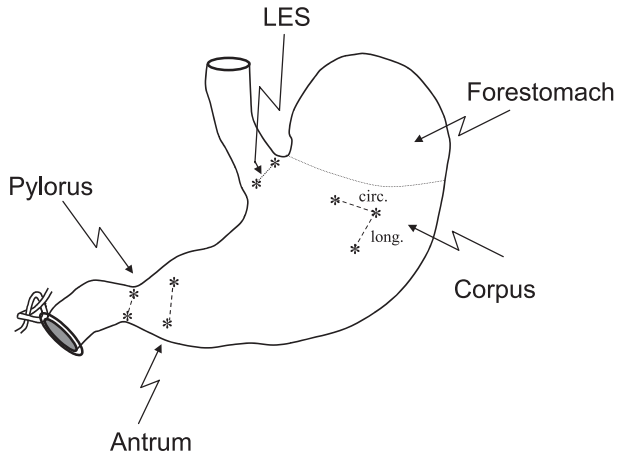

Fig. 1. Schematic representation of piezoelectric crystal positions on the stomach and sphincters. Dashed lines indicate crystal pairs from which distance data were recorded and are not physical structures.

Sonometric distance signals contained two types of noise that were removed via a Spike 2 software script: "digital noise" (Fig. 2*A*) and transient "level shifts." Both of these were characterized by rates of rise 10-fold greater than the fastest signal occurring in the measured signals. The script algorithm located such jumps and then searched for another jump in the opposite direction within a maximum specified period of time, usually 0.2 s, and then replaced the intervening points with a straight line connecting the points on either side of the discontinuity (deglitching). The deglitched sonometric traces (Fig. 2) and the IGP trace exhibited a rhythmic component with a periodicity of ~1.5 Hz in perfect synchrony with the respiratory signal (Fig. 2, *inset*), resulting from the movement of the diaphragm. This respiratory component of the signals was typically most prominent in the LES trace and least well developed in the COR trace. During analysis, it was removed by smoothing the trace with a 1-s smoothing window (Fig. 2*C*).

Phasic and tonic components of the signals were analyzed separately. The tonic component of the trace was obtained by replacing each point in the deglitched smoothed trace with the median value of the trace over the surrounding 10 s. The phasic component was obtained by applying to the original trace the inverse operation of a smoothing function with a 10-s window, i.e., by removing the "DC component" with a time constant of 10 s.

Values of the following variables were calculated for the tonic component of each response period: mean normalized value during the response, 1-min maximum excursion from baseline, duration of response, and integrated response (mean normalized response time duration). Values were normalized by subtracting the respective values during the 3-min basal period before treatment and dividing by the basal value, yielding the percent change from initial conditions. The onset of the tonic response to a stimulus was defined as time at which the tonic component exceeded a threshold of two standard deviations above (for increases) or below (for decreases) the mean value during the 3-min basal period immediately preceding the stimulus. The end of the tonic response was defined as the time at which the trace returned within 10% of the threshold value relative to the 1-min maximum excursion from threshold (90% recovery). A zero value was assigned for any trial in which the trace did not cross the threshold within 3 min of intravenous injection or did not remain beyond the threshold value for at least 45 s. Additionally, the duration and integrated response were not tallied for a given trial if after crossing the threshold the trace remained beyond threshold levels for >30 min (e.g., due to a long-term trend in the trace).

Rate of contraction was determined by marking the peaks or troughs (depending on the region) in the phasic component of the signal. The threshold level for identifying a peak/trough was set at the mean ± 1.5 SD in the 3 min before treatment. Duration of inhibition of phasic activity by CCK-8 was defined by setting as a threshold a 20% reduction in the contractile frequency compared with the 3 min immediately preceding peptide injection. Onset of the inhibition of phasic activity was defined as the center of the first minute during which the contractile frequency fell below half of this threshold value. The end of the response (i.e., recovery of phasic activity) was taken as the beginning of the first minute during which contractile frequency rose above the threshold frequency.

Periodicity of rhythmic contraction after vehicle and devazepide treatment was determined by performing an autocorrelation of the

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

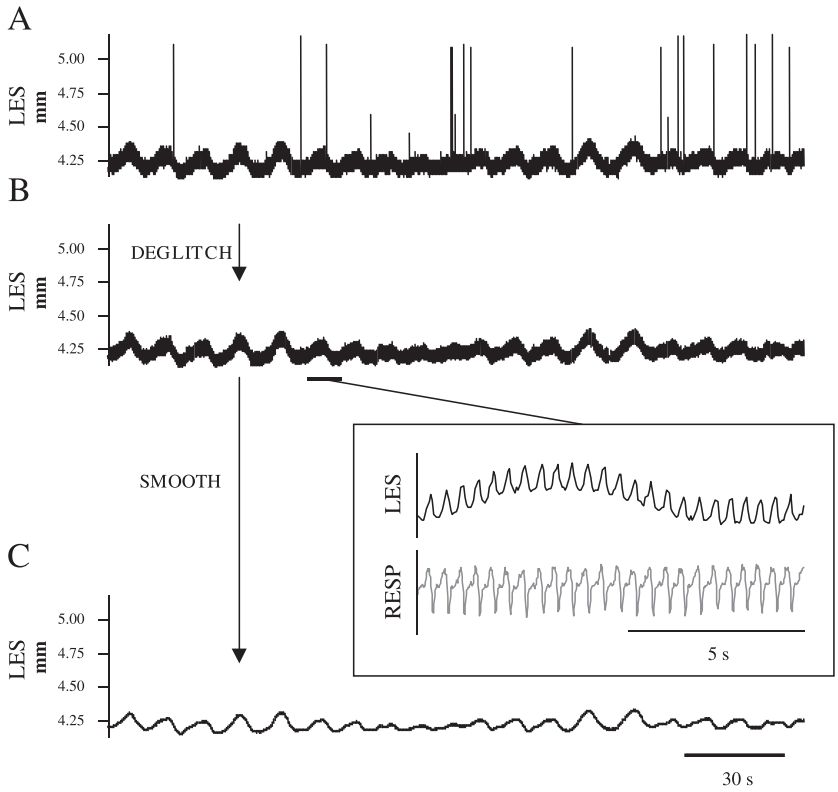

Fig. 2. Signal processing sequence. *A*: raw data trace [lower esophageal sphincter (LES) during basal period] containing "digital noise" transients. *B*: trace "deglitched" by a software script that recognizes and removes transient digital noise and level shifts. *C*: smoothing applied to deglitched trace to remove respiratory rhythm. *Inset*: comparison of LES and respiratory traces. Downward deflection on the respiratory trace indicates inhalation, whereas upward deflection indicates exhalation.

pressure signal for a 5-min interval beginning 25 min after vehicle or devazepide and marking the position of peaks in the autocorrelogram. The amplitude of rhythmic contraction was measured as the root mean square (RMS) amplitude of the phasic component of the trace over the same interval.

Treatments

Sulfated CCK-8 (1 μg/μl in physiological saline; Peninsula Labs, Belmont, CA) was stored at −80°C. Stock solutions were diluted in 0.1% fetal BSA in sterile pyrogen-free phosphate-buffered saline, pH 7.4 (Sigma, St. Louis, MO) immediately before each experiment. Devazepide (L-364718; Merck Sharpe and Dohme, St. Louis, MO) was dissolved in DMSO/Tween 80/saline at a ratio of 1:1:8, respectively, just before administration. All compounds were given intravenously in 0.1 ml and were chased with 0.15 ml of saline.

Experimental Design

After completing the surgery, urethane-anesthetized rats were left undisturbed for 30 min, after which 1 ml of saline was delivered into the stomach via the intragastric cannula. This usually raised IGP initially to 4.0–5.5 cmH_2O. If IGP was below this level, an additional ≤0.5 ml of saline was added. After recording basal levels of all physiological variables for 30 min, vehicle (saline + 0.1% BSA) was injected intravenously followed by consecutive, increasing doses of CCK-8 (0.3, 1, and 3 μg/kg) at 30-min intervals. Thirty minutes after this series of injections, the stomach was distended via intragastric injection of saline in increasing volumes (0.5, 0.7, and 1.0 ml) for 3 min per distension followed by withdrawal of the injected volume and a 3-min rest period.

In a subset of experiments, 30 min after these distensions, the following four injections were made at 30-min intervals: vehicle (10% DMSO:10% Tween 80:80% saline), CCK-8 (3 μg/kg), devazepide (500 μg/kg), and CCK-8 (3 μg/kg). The dose for devazepide was chosen based on a recent study (7).

In an additional set of experiments (n = 4), subdiaphragmatic intraesophageal pressure was also monitored, as well as responses to intravenous injection of vehicle and CCK (3 μg/kg). In these animals, after the placement of the piezoelectric crystals and the closing of the abdominal incision, an additional catheter pressure transducer was placed immediately subdiaphragmatically in the esophagus via the mouth. The subdiaphragmatic position of this catheter was determined by observing the inversion of the excursion in pressure during each respiratory cycle when crossing the diaphragm. Additionally, when initially positioning the intraesophageal pressure transducer, it was pushed through the LES high-pressure zone (LES-HPZ) and then slowly withdrawn to ensure that it was located proximal to the LES-HPZ. The catheter was fixed in position by taping the body of the catheter to the incisors of the rat.

At the end of each experiment, the abdominal cavity was opened and digital photographs were taken to record the crystal placement (Fig. 1), after which animals were euthanized by intracardiac urethane injection followed by section of the abdominal aorta.

Statistics

Unless otherwise noted, all values reported are means ± SE. One-way repeated-measures ANOVA using a post hoc Tukey's test for individual comparisons were used to analyze statistical significance of pressure or motion responses to successive doses of CCK-8 in each region (PYL, ANT, COR, and LES). Values from traces for which data were not obtained for all trials were excluded when calculating statistical significance due to the inability of the test to accommodate missing values. The same test was used to compare durations of response between traces for each treatment. Mean percent reductions in response (relative to vehicle controls) after devazepide were calculated by averaging the percent reduction occurring in each preparation individually. Statistical significance of devazepide-induced reductions in CCK-8-evoked responses was calculated using a one-tailed repeated-measures Student's t-test comparing the response

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

after vehicle to that after devazepide for each preparation. Statistical significance of devazepide-induced changes in amplitude of rhythmic activity was determined using the Wilcoxon signed-rank test. *P* values ≤0.05 were considered significant. All statistical tests were performed using the software program Prism version 3.0 (GraphPad Software, San Diego, CA).

RESULTS

Under basal conditions after inflation of the stomach with ~1 ml saline, mean recorded IGP was 3.9 ± 0.2 cmH_2O (n = 9) and mild rhythmic contractile activity with a periodicity of 5.7 ± 0.3 per minute (n = 9) was observed. These rhythmic events were usually episodic and asymmetric in form, rather than continuous and sinusoidal. During asymmetric events, IGP and LES intracrystal distance (ICD) rose, whereas ANT and PYL ICD typically fell, indicating circumferential shortening. When rhythmic activity could clearly be distinguished in CORci and CORln, ICD increased with each rhythmic event. Saline injection (iv) did not alter either the motion traces or the IGP.

Gastric Responses to intravenous CCK-8

Onset of the response to intravenous CCK-8 occurred immediately, i.e., during injection. The pattern of changes comprised a dose-related tonic reduction in ICD of PYL, ANT, and CORln, opening of LES, and a drop in IGP (Figs. 3 and 4). CORci responses varied in direction (contraction or stretching) between preparations but were consistent for repeated trials within a single preparation. Tonic responses were accompanied in all traces by an inhibition of prevailing rhythmic activity that recovered as the tonic component of the traces returned to baseline levels. The amplitude and frequency of this rhythmic activity were greatest immediately after the end of the tonic response to intravenous CCK-8 and decayed somewhat thereafter, although they remained elevated above prestimulus levels (Figs. 3 and 4). The responses of IGP, PYL, ANT, LES, and COR are described individually below, after which the temporal relationships between traces during CCK responses and during rhythmic contractions are detailed.

IGP. Injection of CCK-8 (0.3, 1, and 3 μg/kg iv) dose-dependently reduced IGP. Duration of response, 1-min maximal drop in IGP, and the integrated response to CCK-8 were significantly different from the vehicle control at all doses (Fig. 4). At 3 μg/kg iv CCK-8, IGP tonic activity and inhibition of phasic activity lasted 6.6 ± 1.1 and 5.2 ± 0.7 min (n = 8), respectively.

PYL and ANT. Injection of CCK-8 (0.3, 1, and 3 μg/kg iv) caused a dose-related contraction of PYL and of ANT, as indicated by a reduction in the ICD within each crystal pair (Figs. 3 and 4). The 1-min maximum of PYL contraction was similar at the 1 and 3 μg/kg doses (−17.1 ± 2.2 vs. −17.9 ± 2.1%) suggesting saturation of the peak response, whereas the period over which the maximum contraction was maintained was significantly greater at 3 than at 1 μg/kg (e.g., Fig. 4*A*). The magnitude of the integrated PYL contractile response to CCK (0.3, 1, and 3 μg/kg) and the duration of response were also dose related and significantly different from the saline response at all doses (Fig. 4, *B* and *C*). At 3 μg/kg, tonic activity and inhibition of phasic activity lasted 9.3 ± 1.2 and 4.2 ± 0.5 min (n = 6), respectively. During the return to basal tone after maximal CCK-8-evoked PYL contraction, we commonly observed one or more abrupt separations and "recontractions" (e.g., Fig. 4*A*).

ANT contractile responses to intravenous CCK-8 were similar to PYL responses, although they were smaller in magnitude and somewhat less consistent in profile (shape) between preparations. Peak and integrated responses were significantly different from vehicle controls only at the highest dose of CCK-8 (3 μg/kg iv) (Fig. 4). At 3 μg/kg, ANT tonic activity and inhibition of phasic activity lasted 6.5 ± 1.1 and 5.3 ± 0.6 min (n = 3), respectively.

LES. Intravenous CCK-8 caused a dose-related separation of LES crystals (LES opening). The peak LES response to CCK-8 was significantly greater than the response to vehicle at all doses of CCK-8 tested, whereas the integrated response showed greater variability and was therefore only significantly different from controls (no response) at 3 μg/kg (Fig. 4). At 3 μg/kg, phasic activity was inhibited for 4.4 ± 0.6 min (n = 9)

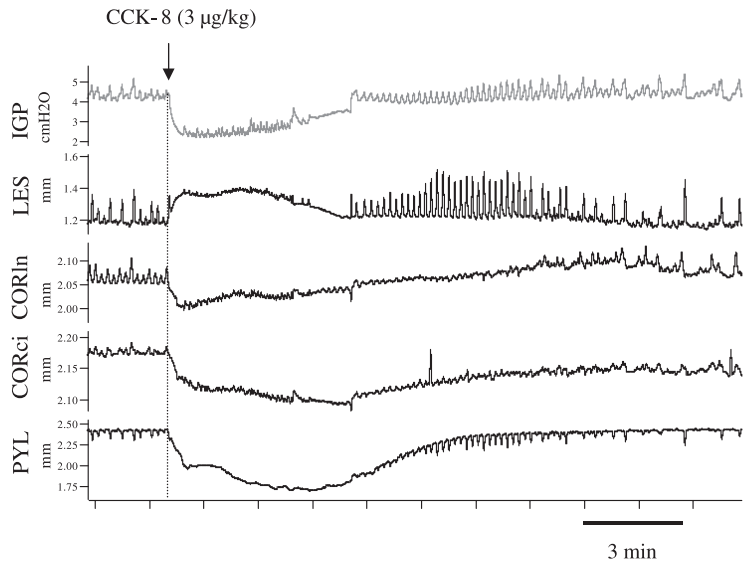

Fig. 3. Pattern of response to intravenous injection of CCK-8 (3 μg/kg iv) in urethane-anesthetized rats. Intragastric pressure (IGP) dropped, as pylorus (PYL), circular corpus (CORci), and longitudinal corpus (CORln) contracted and LES separated. Prevailing rhythmic activity was inhibited during the tonic response and increased in both frequency and amplitude on recovery. Note that LES closed well before PYL reopened to preinjection levels.

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

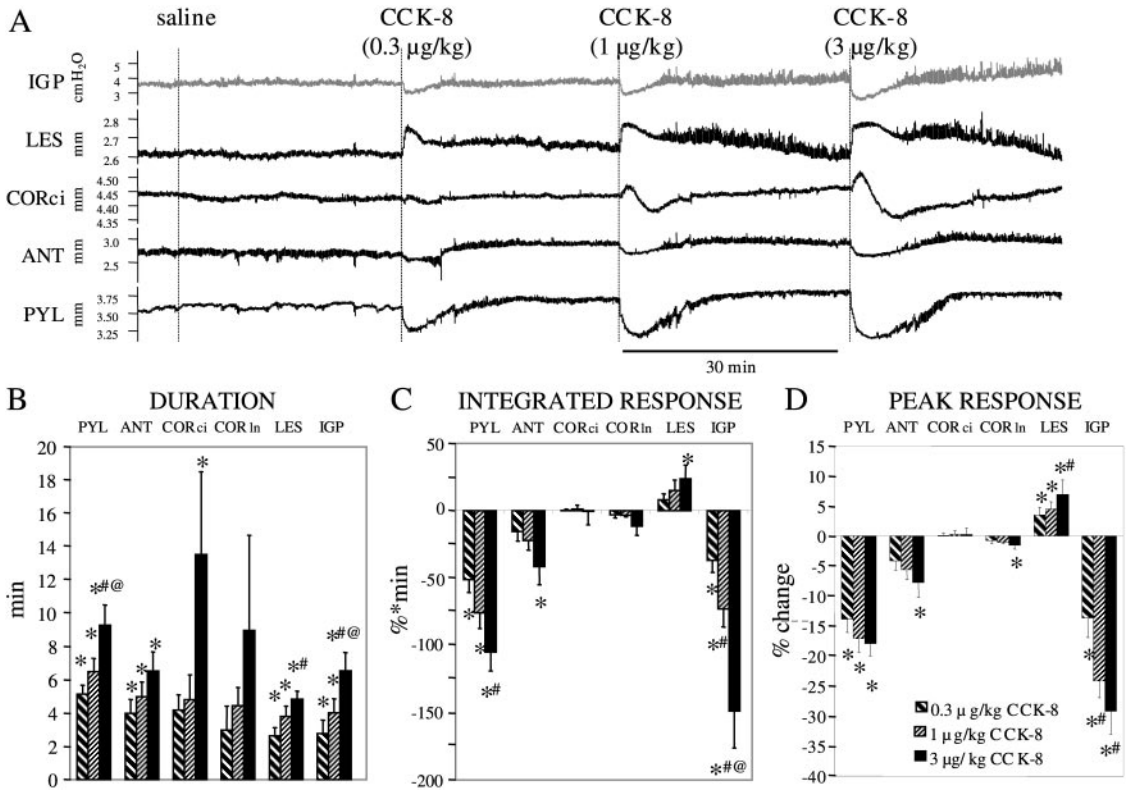

Fig. 4. *A*: dose-related responses to repeated intravenous injection of CCK-8 in a single preparation. Increasing thickness of the traces after intravenous CCK-8 is due to the increase in the amplitude of rhythmic contractions. *B–D*: dose-related duration of response, integrated response, and peak response (1 min maximum) to CCK-8. *$P < 0.05$ relative to saline; #$P < 0.05$ relative to 0.3 μg/kg CCK-8; @$P < 0.05$ relative to 1 μg/kg CCK-8 (repeated-measures ANOVA with Tukey's post test; $n = 3$–9 rats).

and thereafter returned to levels at or above preinjection amplitudes and frequencies essentially concomitant with the return of the tonic LES ICD to baseline levels (Figs. 4 and 5*A*).

Values for tonic duration and integrated response of LES do not include those trials in which the ICD did not return uniformly to within 90% of pretrial baseline levels but in which instead a secondary prolonged plateau occurred in the LES response (Fig. 5*B*). This latter type of opening response, designated a type II response, was observed in two of nine preparations at all doses of CCK-8 tested and in an additional two preparations only at the 3 μg/kg dose. The form of the profile was essentially the same for both the more typical (designated type I) and the type II opening responses (Fig. 5). Type II responses did not differ significantly from the usual LES opening response in terms of 1-min maxima or latency to 1-min maxima [6.9 ± 2.4% at a latency of 2.0 ± 0.1 min (n = 5) for type I vs. 6.0 ± 1.4% at a latency of 1.9 ± 0.9 min (n = 4) for type II at 3 μg/kg] nor duration of inhibition of phasic activity [4.3 ± 0.5 min (n = 5) vs. 4.5 ± 1.3 min (n = 4), respectively]. However, durations of type I and type II tonic responses formed distinct, statistically significantly different populations [4.9 ± 0.5 vs. 18.2 ± 3.1 min at 3 μg/kg (n = 5 and 4), respectively] and thus so too did the integrated responses. Recovery of phasic activity of type II response coincided with the inflection in the shoulder of the tonic response.

To determine whether the separation of the LES crystals was indicative of LES opening, in a separate set of experiments (n = 4) a pressure sensor was placed in the esophagus immediately below the diaphragm. CCK-8 injection (3 μg/kg iv) caused an increase in intraesophageal pressure (Fig. 6). The major rising slope of the immediately subdiaphragmatic intraesophageal pressure often corresponded to the shoulder in the LES crystal separation curve as shown in Fig. 6.

COR. Of the four regions investigated, the amplitude of motional responses of the COR to iv CCK-8 was smallest. COR responses, which were defined as increases or decreases in ICD exceeding two standard deviations of the pretrial mean beginning within 3 min of injection and lasting at least 45 s were observed in all preparations. However, the combination of the small mean amplitude and large variance in amplitude of response resulted in pooled values not significantly different from no response other than for the 1-min peak contraction of CORln. In contrast to the other regions, the CCK-induced changes in CORci were not in the same direction for all preparations. However, the direction of the response (contraction or expansion) was consistent and CCK-8 dose-related for repeated trials within each preparation. In four of seven preparations a reduction of CORci ICDs was observed, whereas in three of seven preparations either separation of CORci crystals or separation followed by contraction occurred (e.g., Fig. 4). In neither group, however, were the aggregate responses significantly different from vehicle controls.

Effect of Devazepide on Motional and Manometric Responses to intravenous CCK-8

Devazepide (500 μg/kg iv) treatment significantly reduced the 1-min maximum response, the duration of response, and the

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

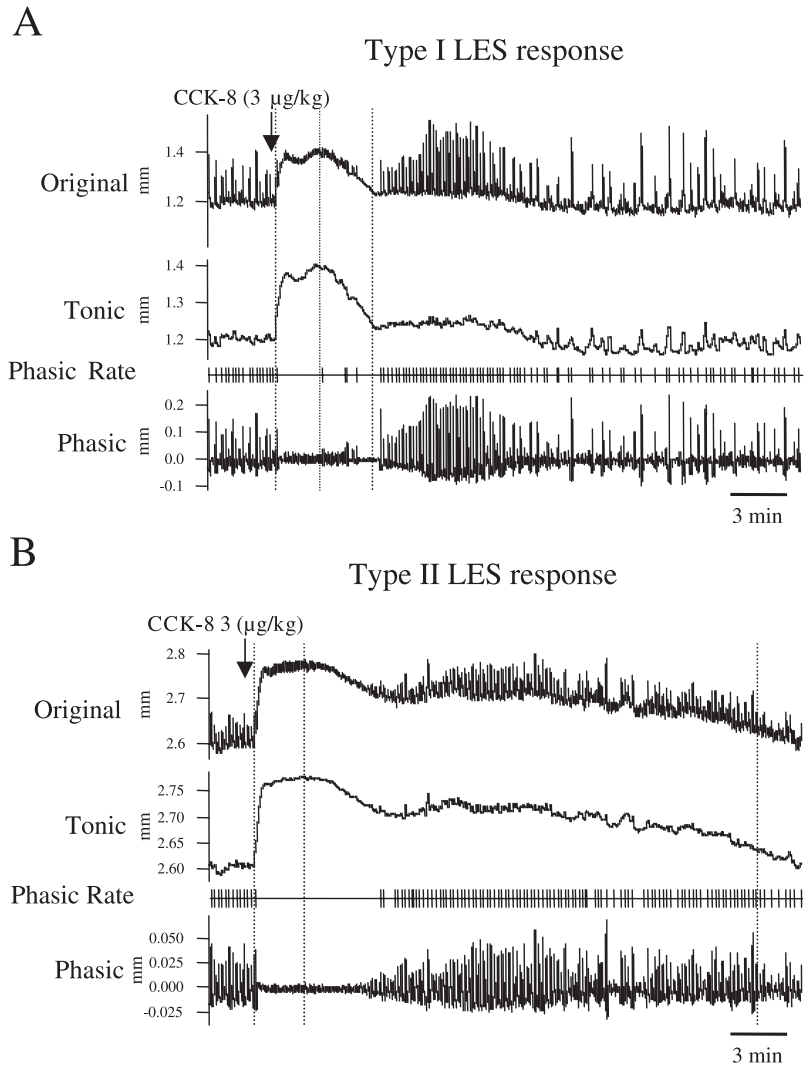

Fig. 5. LES responses to CCK-8 (3 μg/kg iv). *A–B*: the original LES trace, the separated tonic and phasic components of the trace, and a marker trace indicating the rate of phasic activity. Vertical dotted lines indicate calculated onset, peak, and recovery times on the basis of the tonic trace. *A*: type I response; *B*: type II response.

integrated response of ANT, PYL, and LES to CCK-8 (3 μg/kg iv) relative to the response to CCK-8 after vehicle (Fig. 7). Devazepide also significantly reduced both the duration of the CCK-8 (3 μg/kg iv)-evoked drop in IGP and the integrated IGP values by 64 ± 6.6 and 36 ± 19.8%, respectively ($n = 6$, Fig. 7). However, devazepide did not reduce the 1-min maximum drop in IGP induced by CCK-8 (Fig. 7, *B* and *E*).

Devazepide itself increased the RMS amplitude of rhythmic contraction in all preparations (0.20 ± 0.03 cmH_2O 25 min after vehicle vs. 0.61 ± 0.26 cmH_2O 25 min after devazepide, $n = 6$), whereas it had no effect on the periodicity of rhythmic contraction in any preparation. Devazepide also significantly increased the baseline IGP, from 5.3 ± 0.3 cmH_2O 25 min after vehicle to 7.2 ± 0.5 cmH_2O 25 min after devazepide ($n = 6$).

Temporal and Quantitative Relationships Between Responses

Onsets of response to CCK-8 for PYL, ANT, and LES motion traces and changes in IGP pressure were simultaneous (Fig. 8*A*). Latencies from the onset of response to the maximal excursion of the tonic response demonstrated that the maximal drop in IGP preceded the maximal excursion in all motion traces at all doses, with the exception of the LES trace at the 0.3 μg/kg dose of CCK-8 (Table 1).

Tonic response durations measured for each trace were compared with all other simultaneously recorded traces to determine whether any consistent relationships exist. Statistically significant differences were found in the durations of PYL and LES responses but not between any other pairs of traces (repeated-measures ANOVA with post hoc Tukey's test; for the purposes of this comparison, only type I LES responses were considered). In 18 of 18 trials from 7 experiments, the PYL response lasted longer than the LES response ($P < 0.001$). This difference reflected the clearly visible more rapid return toward baseline levels from the peak response of LES compared with that of PYL (Fig. 8*B*). The difference in duration of response of PYL relative to LES was 2.4 ± 0.7, 2.0 ± 0.5, and 3.3 ± 1.2 min after CCK injection at 0.3, 1, and 3 μg/kg, respectively. In contrast to the case for tonic responses, no statistically significant difference was found between the times of recovery of phasic activity in the various traces. Increases in the amplitude of rhythmic contractions in all regions often occurred coincident with the apparent closure of the LES (e.g., Figs. 3, 4, and 6).

Responses to Gastric Distension

Gastric distension (0.5, 0.7, and 1.0 ml) reliably caused marked stretching of CORci [2.0 ± 0.4 ($n = 4$), 2.9 ± 1.1 ($n =$

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

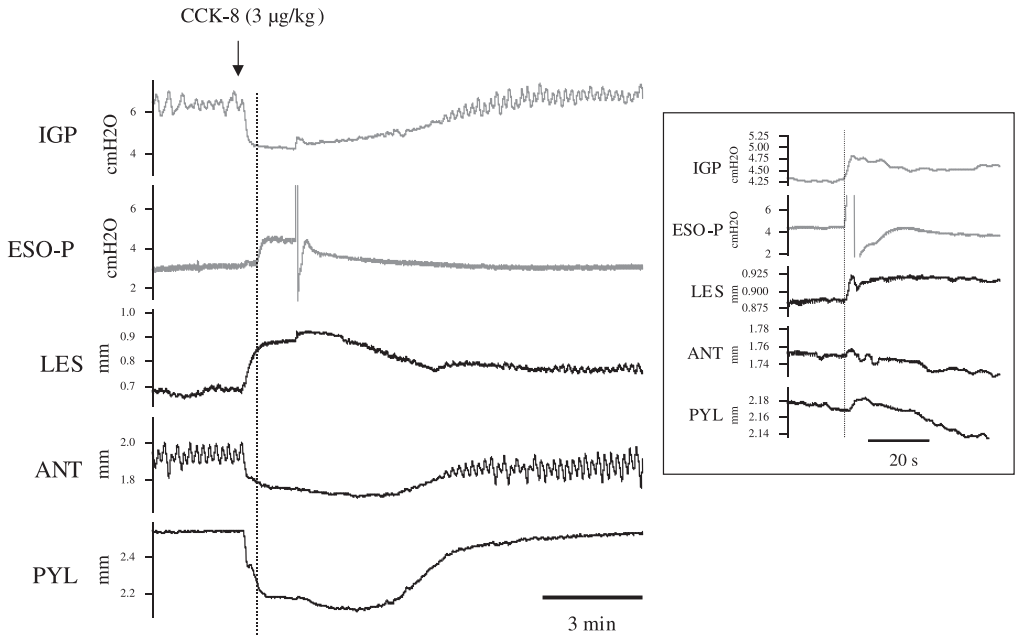

Fig. 6. Subdiaphragmatic esophageal pressure (ESO-P) rises after intravenous CCK-8 injection, suggesting formation of a common cavity between stomach and subdiaphragmatic esophagus. The arrow indicates the time of injection, whereas the dotted vertical line marks the onset of a rapid rise in ESO-P measured immediately below the diaphragm, which coincides with the shoulder in the LES trace. The large transient in ESO-P midway through the response was accompanied by a simultaneous small rise in IGP, along with small increases in LES, ANT, and PYL intracrystal distances (*inset*: vertical dotted line marks onset of esophageal pressure transient).

5), and 3.5 ± 1.0% (n = 8), respectively] and increased IGP [32.9 ± 75.5 (n = 6), 46.5 ± 7.2 (n = 8), and 60.1 ± 8.2% (n = 8), respectively]. This stretching of CORci occurred in all preparations, regardless of the direction of response to CCK-8, and the magnitude of the response was greater by more than a factor of 10 than the maximal response to CCK-8. Responses of other regions (PYL, ANT, and LES) varied between preparations, although within a given animal they were dose related and consistent in direction for repeated trials (Fig. 9).

DISCUSSION

The present study shows that digital ultrasonomicrometry provides an effective way to measure simultaneous motional changes in multiple sites without extensive gastric surgical manipulation and is particularly useful in the study of sphincter motility. Ultrasonometric measurements can detect muscle shortening or lengthening, both tonic and phasic, and permit discrimination of longitudinal and circular movements. It should be emphasized that observation of a change in ICD does not provide information on the associated changes in force developed within the muscle.

The present ultrasonomicrometry and manometry data demonstrated a consistent pattern of response to intravenous CCK-8 that includes contraction of PYL and ANT, simultaneous with opening of the LES and a drop in IGP. Inhibition of prevailing rhythmic contractions occurred concomitantly. Effects of intravenous CCK-8 on gastric motion were significantly inhibited by pretreatment with the CCK_A receptor antagonist devazepide, demonstrating that the responses are CCK_A-receptor mediated. These observations are consistent with a number of prior reports in rats and other species that have investigated separately either pyloric, antropyloric, LES, and/or IGP responses to intravenous CCK (1, 2, 18–21). The present sonomicrometric data extend prior observations by permitting comparison of the temporal relationships between motility changes in different portions of the stomach and a comparison of this motion with IGP changes. The most significant observation of the present study concerns the coordinated activity of LES and PYL.

PYL and ANT

Intravenous CCK-8 caused significant reduction of PYL and ANT tonic ICD. Circumferential shortening of the muscle could be accompanied by maintenance of constant tension during a drop in IGP (resulting from accommodation occurring in more proximal parts of the stomach), decreases in wall tension offset by greater decreases in the opposing force of IGP, or increases in wall tension, i.e., active contraction occurring simultaneously with the drop in IGP. Prior studies using sleeve manometry, force measurements, or electromyography (1) support the view that the circumferential shortening observed in PYL and ANT results from active contraction (11, 12, 14, 23). Pyloric contractile responses were more consistent in shape and were larger in magnitude (as a percent change from preinjection values) than changes occurring in the other gastric regions monitored. Consistency of response between preparations may be due, in part, to the small size of the PYL and the reliability with which crystals could be placed at the same position in every preparation due to the clear anatomic landmarks. Furthermore, the functional role of the PYL is to constrict to closure, and the movements of the PYL are thus likely to be simpler than movements in the body of the stomach, which can include longitudinal, circular, and oblique components. ANT responses were typically similar to pyloric

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

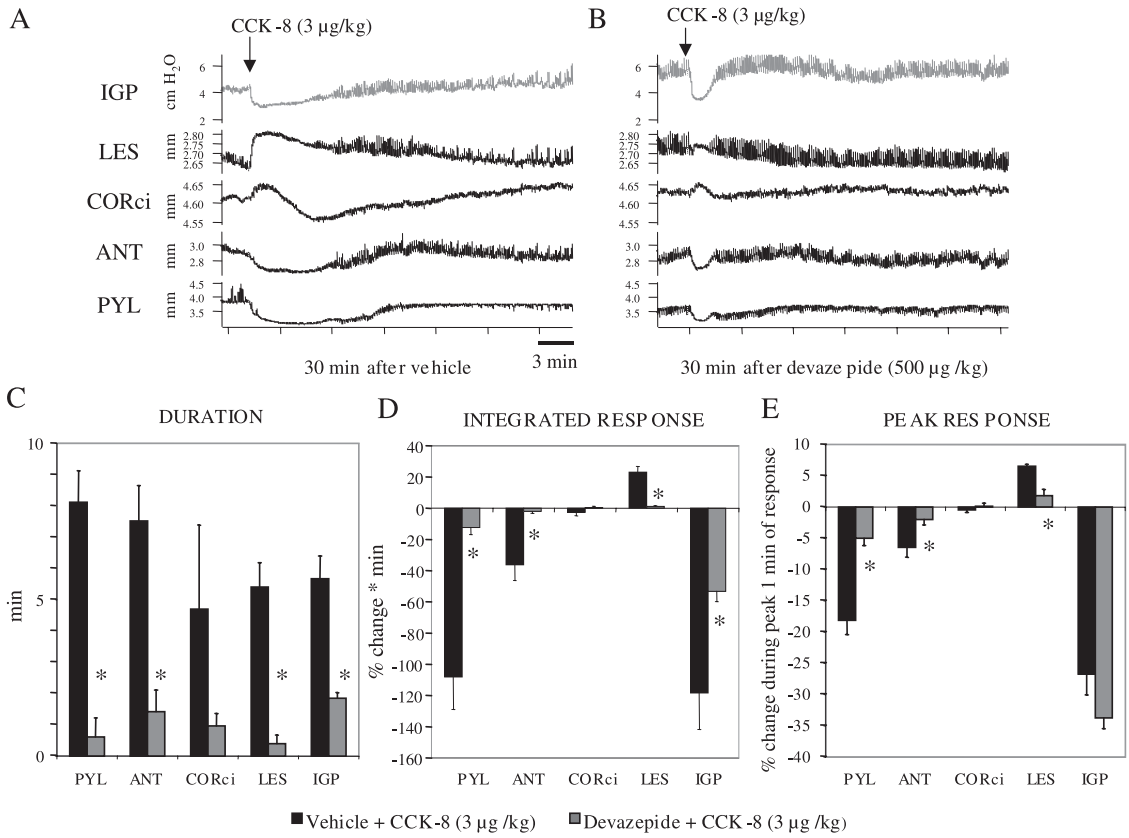

Fig. 7. Devazepide pretreatment inhibits motional responses to intravenous CCK-8 in all traces and reduces the integrated magnitude and duration of the IGP response. However, the peak IGP drop is not reduced. *A*: CCK-8 response 30 min after intravenous vehicle injection. *B*: CCK-8 response in the same preparation 30 min after devazepide (500 μg/kg iv). *C–E*: response duration, integrated response, and peak response to CCK-8 (3 μg/kg iv) for vehicle + CCK-8 and devazepide + CCK-8 (n = 3–6 rats). *$P < 0.05$ relative to vehicle + CCK-8.

responses, although they were smaller in magnitude and somewhat less consistent in profile between preparations. Pyloric and ANT responses to intravenous CCK-8 consisted of tonic contraction accompanied by inhibition of phasic activity followed by a recovery to baseline tone, during which phasic activity also recovered to levels that exceeded prestimulus amplitude and frequency. In some preparations, the recovery of PYL to baseline tone was interrupted by a sharp return to the baseline level followed immediately by recontraction (e.g., Fig. 4). The pyloric ultrasonometric profile was remarkably similar, if not identical, to a published trace of strain gauge recordings of isolated pyloric sphincter responses to bath application of CCK-8 in vitro (18). This marked similarity between the features of the in vitro strain gauge and in vivo sonometric recordings suggests that the pathways responsible are intrinsic to the pyloric smooth muscle.

LES

In response to CCK-8, the LES ICD increased significantly, suggesting opening of the LES. Visual inspection of the traces suggested that in most cases increased amplitude of phasic activity coincided closely with recovery of initial LES ICD. This was reflected in the greatest similarity between the duration of the tonic response and the duration of phasic inhibition in the LES compared with other regions. Intraesophageal pressure measurements indicated that separation of LES crystals was accompanied by creation of a common cavity between stomach and subdiaphragmatic esophagus, presumably due to relaxation of the LES and the presence of a pressure gradient between the stomach and esophagus. Relaxation of the LES in response to intravenous CCK-8 has been demonstrated in the cat (2). An intact nerve supply is necessary because tetrodotoxin pretreatment reverses this effect leading to LES contraction in response to intravenous CCK-8 (2). Similarly, a variety of human studies indicate that exogenous (13) or endogenous, postprandially released CCK (25, 28) causes LES relaxation, whereas CCK-8 contracts isolated human LES muscle strips in vitro (8). In contrast, in the opossum intravenous CCK-33 causes LES closure and an increase in IGP (6). To the best of our knowledge, the present study provides the first demonstration in rats of LES opening in response to intravenous CCK.

COR

CORci was unique among the motional traces in that CCK-8-evoked changes varied in direction (expansion or contraction) in different preparations, although within a single preparation the direction of response was consistent for repeated trials. Due to the variability in the magnitude of response and the small amplitude of response, as a group, the amplitudes of CORci responses (1-min maximum and integrated response) did not differ significantly from zero response. A contributing factor to the small amplitude of CORci ICD changes may be the much larger

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

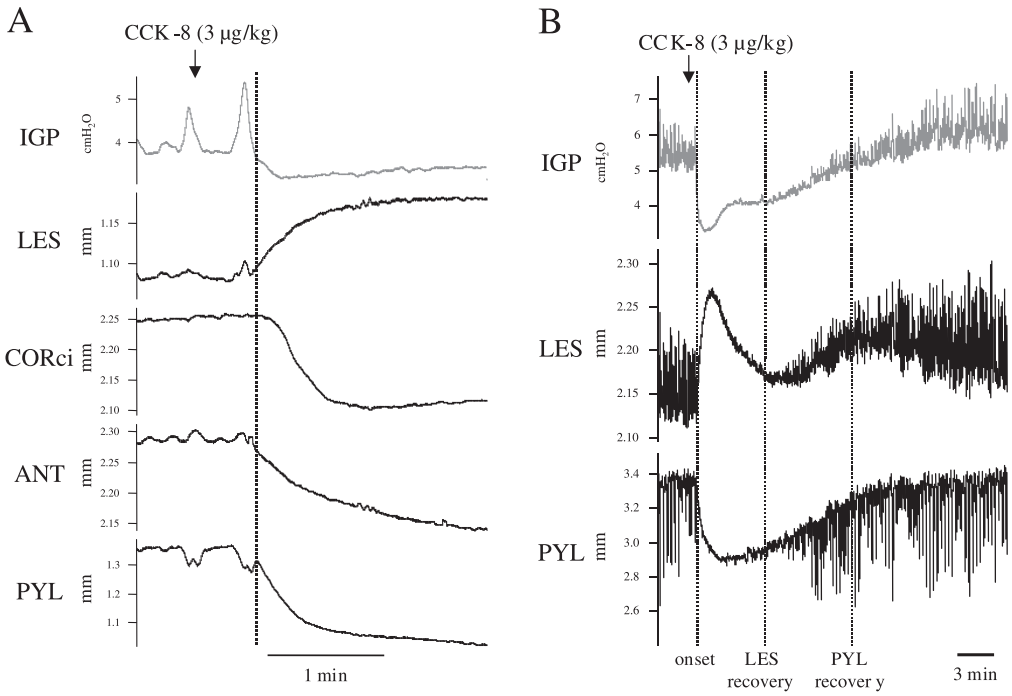

Fig. 8. Temporal relationships between traces during responses to intravenous CCK-8 (3 μg/kg). *A*: onset of response for PYL, ANT, and LES motion traces and for the change in IGP pressure were simultaneous. CORci responses appear to begin up to 10 s after that in other traces. *B*: LES returns to baseline levels (reclosure) before PYL (reopening). Vertical dotted lines indicate software-determined onset of response (both LES and PYL), recovery of LES (90% of pre-CCK-8 tonic level), and recovery of PYL (90% of pre-CCK-8 tonic level).

circumference of the stomach at its midpoint than in the hindstomach, such that smaller percent changes in midstomach circumference displace greater volume than do larger percent changes in hindstomach circumference. Because three of three CORln responses and four of seven CORci responses to CCK-8 were contractions, it appears that most of the volume displaced by hindstomach contraction is accommodated in the forestomach of the rat stomach in this preparation and not in the midstomach region. If, in response to intravenous CCK-8, the hindstomach contracts circumferentially, whereas accommodation occurs in the forestomach, at some point along the longitudinal axis of the stomach there will be a region that is transitional between circumferential contraction and circumferential expansion, and there must be a position in this transition zone in which there is no net change in circumference. It is possible that the site we selected for measuring circumferential COR movements is positioned in such a transition zone, leading to both the small amplitudes of response observed and to the variability in the direction of response between preparations. Further work using more crystals placed in this region will be necessary to determine whether this is indeed the case.

Table 1. *Latencies to maximum response to intravenous CCK-8*

	CCK, μg/kg		
	0.3	1	3
LES	1.2±0.4†	1.4±0.4*‡	2.0±0.1*†
COR	2.1±0.4*	1.8±0.5*	4.7±1.3*
ANT	1.7±0.7*	1.7±0.3*	1.9±0.6*†
PYL	1.5±0.2*†	2.1±0.2*	2.7±0.3*†
IGP	1.0±0.2†	0.9±0.1†	0.9±0.1†

Values are means ± SE in minutes. *Significantly different from intragastric pressure (IGP) ($P < 0.05$, $n = 5$–9); †significantly different from circular corpus (CORci); ‡significantly different from pyloric (PYL); LES, lower esophageal sphincter; ANT, antrum.

Our finding of variability in the direction of COR motion in response to intravenous CCK-8 is similar to the findings of Takahashi and Owyang (24), who measured COR motility using strain gauges sutured to the body of the stomach parallel to the circular muscle of ketamine/xylazine-anesthetized rats. They observed variable responses (contraction, relaxation, and biphasic responses or no response) to intravenous CCK-8 that were influenced by adrenergic antagonists. After α-receptor blockade, intravenous CCK-8 relaxed the stomach body in 40 of 40 rats, whereas after β-receptor blockade, intravenous CCK-8 contracted the stomach body in 7 of 8 rats tested. These authors (24) hypothesized that the variability they observed might result from differences in the balance of autonomic activation in different preparations.

The small CORci changes in response to intravenous CCK-8 contrasted with the large CORci changes in response to distension. In all preparations, including those in which CORci contracted in response to CCK-8, intragastric injection of saline (0.5–1 ml) caused stretching of the both CORci and CORln, and the magnitude of the median response of CORci to distension was 10–15 times greater than the maximum response to CCK-8 for those trials in which CORci stretched.

Relationships Between Traces

The onset of response for all motion traces and for IGP was essentially simultaneous. The overall pattern of response com-

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

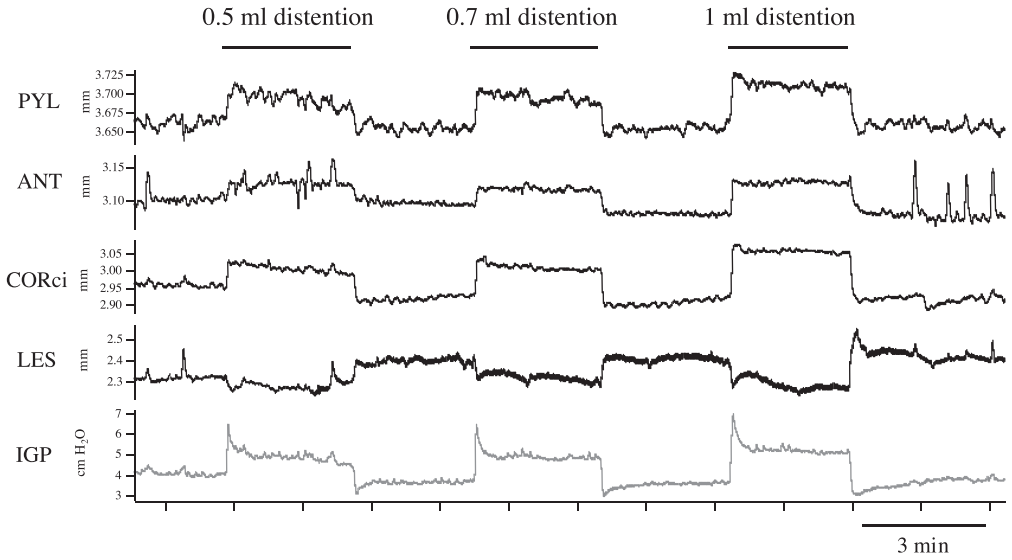

Fig. 9. Responses to successive 3-min distension with 0.5, 0.7, and 1.0 ml saline intragastrically in urethane-anesthetized rats.

prised pyloric and hindstomach contraction, LES opening, and minimal COR movement, concomitant with a drop in IGP. This pattern of activity is consistent with retropulsion of gastric contents away from the pyloric region, in keeping with the physiological role of CCK in delaying gastric emptying (27). The maximal drop in IGP occurred significantly sooner than the maximal tonic motional response in any other region. IGP reflects an integral of activity in all regions of the stomach, including forestomach accommodation, LES relaxation, and hindstomach contraction. Although we did not measure forestomach motion in our experiments, other studies indicate that CCK-8 causes forestomach accommodation, i.e., an increase in forestomach volume (15, 19, 26). The time at which the maximal drop in IGP occurs may coincide with the point of maximal forestomach accommodation, or it may represent the point at which further volume increases in the forestomach are offset by corresponding reductions in hindstomach volume. Venting of pressure through the relaxed LES may also contribute to reduce the IGP.

Duration of response did not differ significantly between any pair of traces, with the exception of PYL and LES. LES reclosure consistently occurred more rapidly than PYL reopening after CCK-8 injection. LES typically appeared to close approximately midway during the return of PYL from maximum contraction toward pre-CCK-injection levels.

Consistency of the relationship between LES and PYL suggests a potentially important physiological principle, namely that in general both sphincters should not be open at the same time. Simultaneous patency of both the gastroesophageal and the gastroduodenal openings would make it difficult to control the pressure gradients across each sphincter and thus difficult to control the direction of flow of material through them. It may be speculated that improper coordination of the activity of the two sphincters could be responsible for coincidence of gastroduodenal and gastroesophageal reflux in some patients (16). If this were the case, investigation of either PYL or LES function alone might not reveal an obvious flaw. Instead, the problem might arise from dysregulation of the timing of action of each relative to the other. In discussing the relationship of the PYL to the LES activity, Bremner (4) has commented that, "although the two sphincters have a relationship in disease, the physiological association has not been clearly demonstrated." To our knowledge, this study is the first in which both gastric sphincters were monitored simultaneously. Further ultrasonomicrometry studies may aid in providing the necessary physiological data to understand a variety of gastric motility disorders.

Effects of Devazepide on CCK-8 Responses

CCK_A receptors are believed to play the predominant role in CCK-mediated effects on gastric motility, including nutrient-evoked inhibition of gastric emptying (3, 19). The CCK_A receptor antagonist devazepide inhibited CCK-evoked motion in all regions measured. However, although the 1-min maximum motion response was significantly reduced in all motion traces, devazepide did not reduce the magnitude of the 1-min maximum drop in IGP in response to CCK-8. This may be due to the combination of several factors. The peak drop in IGP occurs before the peak motional changes in the regions we measured. The inhibitory effects of the competitive antagonist are most successfully overcome at the initial postinjection peak serum concentrations of CCK-8. It is also possible that EC_{50} for forestomach accommodation is lower than that for motional changes in the regions we measured. Concomitant reduced hindstomach contraction may offset the effect of reduced forestomach accommodation on falling IGP. Finally, although the duration and magnitude of LES motion are reduced by devazepide, it remains possible that a common cavity between stomach and esophagus might still be created briefly during the early part of the response to CCK-8 after devazepide. The effect on percentage drop in IGP due to the common cavity would be enhanced by the relatively higher basal IGP after devazepide pretreatment compared with that after vehicle treatment.

Methodological Considerations

Ultrasonomicrometry allows measurement of the straight-line path between piezoelectric crystals affixed to soft tissue

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

and offers a number of advantages for the study of GI motility. It provides a direct means of detecting motion. It combines the high spatial resolution of electromyography (EMG) and strain-gauge measurements with the capacity to detect either expansion or contraction, to track both tonic and phasic components of motion, and to distinguish circular and longitudinal axes of motion. It is suitable for use in small animals and on small structures such as sphincters, and can be applied to simultaneously monitor the activity of adjacent and nonadjacent structures. The placement of crystals on the serosal surface of GI tissues using cyanoacrylate glue for acute experiments is minimally invasive by comparison to the methods for placement of EMG electrodes or strain gauges. Sonomicrometry measurements can be made in fed or fasted animals, and the ease with which crystals can be placed at multiple locations makes it suitable for studying the organization of activity across large expanses of the GI tract. Sonometric signals can also be used to perform three-dimensional (3-D) reconstructions, thus allowing the measurement of enclosed volume and, in combination with simultaneous pressure measurements, of compliance.

In considering the interpretation of sonometric data, several issues must be kept in mind. The data obtained do not provide information on the intramuscular forces giving rise to the observed motional changes. Care must be used in crystal placement. It is important to have consistent landmarks to allow consistent crystal placement between preparations. Also, although in this paper we have used the terms circular and longitudinal to describe the orientation of the crystal pairs, which we placed in similar positions in each preparation, these designations are likely too simplistic in the GI system. The terms assume that crystals are oriented precisely parallel to the respective muscle layers, which in practice is never fully achievable. But more importantly, changes in muscle length need not be uniform along the entire circumference along which a pair of circularly oriented crystals is placed, nor along all parallels to a longitudinally oriented pair of crystals. The stomach has both a greater and lesser curvature, and motion along or across these curvatures may not be identical under all conditions and in fact is unlikely to be so. Furthermore, the stomach is invested with an oblique muscle layer, and the state of oblique muscle tone when placing crystals may influence the initial apparent orientations. Given the potential complexity of the motions of the stomach suggested by the vast intrinsic neuronal network and the multiple muscle layers, as well as the impacts of studying a 3-D motion using two-dimensional measurements, ultrasonometric data must be interpreted with care. In the case of complex motions, the use of a number of crystals in the region of interest and possibly the use of 3-D reconstruction methods may be necessary. However, taking into account these caveats, ultrasonomicrometric measurements can provide valuable information on the coordinated motion of the GI tract and should prove to be a powerful complement to methods such as manometry and EMG recording.

In summary, the ultrasonometric recordings demonstrate a consistent, dose-related pattern of gastric responses to intravenous CCK-8, comprising tonic contraction of PYL and ANT, opening of the LES, and minimal motion of the COR near the dividing line between the nonglandular and glandular portions of the stomach, midway between the greater and lesser curvatures. The simultaneity of the measurements permitted discrimination of a consistently more rapid reclosure of LES than reopening of PYL after CCK-8. This pattern of response may have physiological relevance for avoiding simultaneous continuity of the gastric lumen with both the esophageal and duodenal lumens, thus permitting appropriate control of the direction of flow across each sphincter. These observations may have implications for understanding reflux pathologies. Given its methodological strengths and its usefulness in complementing existing techniques for monitoring GI function, it is likely that the use of ultrasonomicrometry in the study of GI motility will increase in coming years.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-41301 (to Y. Taché, Animal Core) and DK-57238-01A1S1 (to M. Million) and a Veterans Affairs Merit Award (to Y. Taché).

REFERENCES

1. **Allescher HD, Daniel EE, Fox JE, Kostolanska F, and Rovati LA.** Effect of the novel cholecystokinin receptor antagonist CR-1392 on cholecystokinin-induced antroduodenal and pyloric motor activity in vivo. *J Pharmacol Exp Ther* 251: 1134–1141, 1989.
2. **Behar J and Biancani P.** Effect of cholecystokinin-octapeptide on lower esophageal sphincter. *Gastroenterology* 73: 57–61, 1977.
3. **Boulant J, Fioramonti J, Dapoigny M, Bommelaer G, and Bueno L.** Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distention in dogs. *Gastroenterology* 107: 1059–1066, 1994.
4. **Bremner CGDJ.** Inseparable sphincters. Interrelationship between the pyloric and the lower oesophageal sphincters. *S Afr J Surg* 27: 119–124, 1989.
5. **Chang RS and Lotti VJ.** Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. *Proc Natl Acad Sci USA* 83: 4923–4926, 1986.
6. **Dent J, Dodds WJ, Hogan WJ, Arndorfer RC, and Teeter BC.** Effect of cholecystokinin-octapeptide on opossum lower esophageal sphincter. *Am J Physiol Gastrointest Liver Physiol* 239: G230–G235, 1980.
7. **Gay J, Fioramonti J, Garcia-Villar R, and Bueno L.** Enhanced intestinal motor response to cholecystokinin in post-*Nippostrongylus brasiliensis*-infected rats: modulation by CCK receptors and the vagus nerve. *Neurogastroenterol Motil* 13: 155–162, 2001.
8. **Gonzalez AA, Farre R, Mones J, Capella G, and Clave P.** Pharmacological and molecular characterization of muscular cholecystokinin receptors in the human lower oesophageal sphincter. *Neurogastroenterol Motil* 12: 539–546, 2000.
9. **Hellstrom PM and Naslund E.** Interactions between gastric emptying and satiety, with special reference to glucagon-like peptide-1. *Physiol Behav* 74: 735–741, 2001.
10. **Holzer HH, Turkelson CM, Solomon TE, and Raybould HE.** Intestinal lipid inhibits gastric emptying via CCK and a vagal capsaicin-sensitive afferent pathway in rats. *Am J Physiol Gastrointest Liver Physiol* 267: G625–G629, 1994.
11. **Isenberg JI and Csendes A.** Effect of octapeptide of cholecystokinin on canine pyloric pressure. *Am J Physiol* 222: 428–431, 1972.
12. **Katschinski M, Schirra J, Begliner C, Langbein S, Wank U, D'Amato M, and Arnold R.** Intestinal phase of human antro-pyloro-duodenal motility: cholinergic and CCK-mediated regulation. *Eur J Clin Invest* 26: 574–583, 1996.
13. **Ledeboer M, Masclee AA, Batstra MR, Jansen JB, and Lamers CB.** Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. *Gut* 36: 39–44, 1995.
14. **Margolis RL, Moran TH, and McHugh PR.** In vitro response of rat gastrointestinal segments to cholecystokinin and bombesin. *Peptides* 10: 157–161, 1989.
15. **Mesquita MA, Thompson DG, Troncon LE, D'Amato M, Rovati LC, and Barlow J.** Effect of cholecystokinin-A receptor blockade on lipid-induced gastric relaxation in humans. *Am J Physiol Gastrointest Liver Physiol* 273: G118–G123, 1997.

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

16. **Moraes-Filho J, Cecconello I, Gama-Rodrigues J, Castro L, Henry MA, Meneghelli UG, and Quigley E.** Brazilian consensus on gastro-esophageal reflux disease: proposals for assessment, classification, and management. *Am J Gastroenterol* 97: 241–248, 2002.
17. **Moran TH, Kornbluh R, Moore K, and Schwartz GJ.** Cholecystokinin inhibits gastric emptying and contracts the pyloric sphincter in rats by interacting with low affinity CCK receptor sites. *Regul Pept* 52: 165–172, 1994.
18. **Murphy RB, Smith GP, and Gibbs J.** Pharmacological examination of cholecystokinin (CCK-8)-induced contractile activity in the rat isolated pylorus. *Peptides* 8: 127–134, 1987.
19. **Raybould HE.** Capsaicin-sensitive vagal afferents and CCK in inhibition of gastric motor function induced by intestinal nutrients. *Peptides* 12: 1279–1283, 1991.
20. **Raybould HE, Roberts ME, and Dockray GJ.** Reflex decreases in intragastric pressure in response to cholecystokinin in rats. *Am J Physiol Gastrointest Liver Physiol* 253: G165–G170, 1987.
21. **Raybould HE and Tache Y.** Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. *Am J Physiol Gastrointest Liver Physiol* 255: G242–G246, 1988.
22. **Rushmer RF, Franklin DL, and Ellis RM.** Left ventricular dimensions recorded by sonocardiometry. *Circ Res* 4: 684–688. 1956.
23. **Scheurer U, Varga L, Drack E, Burki HR, and Halter F.** Measurement of cholecystokinin octapeptide-induced motility of rat antrum, pylorus, and duodenum in vitro. *Am J Physiol Gastrointest Liver Physiol* 244: G261–G265, 1983.
24. **Takahashi T and Owyang C.** Mechanism of cholecystokinin-induced relaxation of the rat stomach. *J Auton Nerv Syst* 75: 123–130, 1999.
25. **Trudgill NJ, Hussain FN, Moustafa M, Ajjan R, D'Amato M, and Riley SA.** The effect of cholecystokinin antagonism on postprandial lower oesophageal sphincter function in asymptomatic volunteers and patients with reflux disease. *Aliment Pharmacol Ther* 15: 1357–1364, 2001.
26. **Van Der Schaar PJ, Bremer Y, Lamers CB, and Masclee AA.** Role of cholecystokinin in relaxation of the proximal stomach. *Scand J Gastroenterol* 36: 361–366, 2001.
27. **Yamagishi T and Debas HT.** Cholecystokinin inhibits gastric emptying by acting on both proximal stomach and pylorus. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 234: E375–E378, 1978.
28. **Zerbib F, Bruley D, Scarpignato VC, Leray V, D'Amato M, Roze C, and Galmiche JP.** Endogenous cholecystokinin in postprandial lower esophageal sphincter function and fundic tone in humans. *Am J Physiol Gastrointest Liver Physiol* 275: G1266–G1273, 1998.

Downloaded from http://ajpgi.physiology.org/ by guest on May 7, 2013

Copyright of American Journal of Physiology: Gastrointestinal & Liver Physiology is the property of American Physiological Society and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.