

Correlation Between Gallbladder Size and Release of Cholecystokinin After Oral Magnesium Sulfate in Man

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In order to determine the effect of oral magnesium sulfate on gallbladder contraction and release of cholecystokinin (CCK) in man, magnesium sulfate (25 g in 100 ml distilled water) was given by mouth to five fasting adult male volunteers. Plasma samples were collected for measurement of CCK by a specific radioimmunoassay. Gallbladder volumes were determined from sonograms obtained from a phased-array real-time ultrasound scanner. Basal concentrations of CCK (82.2 ± 10.1 pg/ml) increased significantly at 20 minutes after oral magnesium sulfate (113.8 ± 7.1 pg/ml), and reached a maximal value at 50 minutes (150.0 ± 42.0 pg/ml). The mean basal volume of the gallbladder was 30.8 ± 5.3 cm³ and maximum reduction of gallbladder volume (to one third of original) was achieved at 50 minutes after ingestion of magnesium sulfate. Linear regression analysis showed a close correlation ($r = -0.9337$) between plasma concentrations of CCK and gallbladder size in response to magnesium sulfate. Oral magnesium sulfate also caused a significant increase in serum gastrin (from basal of 51.4 ± 9.9 pg/ml to 69.8 ± 15.5 pg/ml at 5 min); there was no significant correlation between gastrin release and gallbladder contraction. This study provides direct evidence that the mechanism of magnesium sulfate-stimulated gallbladder contraction occurs through the release of CCK, and shows a close correlation between CCK release and contraction of the gallbladder.

MAGNESIUM SULFATE is a potent stimulant of gallbladder contraction, as measured by bioassay, measured either by bile flow,²⁻⁷ or by cholecystography.^{3-5,8,9} The mechanism for this stimulation has not been proven. Since Ivy and Oldberg's first report concerning cholecystokinin (CCK),¹⁰ stimulation of gallbladder contraction has been recognized as a major physiologic effect of CCK.

Presented in part at the American Gastroenterological Association Meeting held in New York, May 1981 (1).

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Supported by grants from the National Institutes of Health (AM 15241) and the Moody Foundation (#81-57).

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Submitted for publication: October 18, 1982.

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The purpose of this study was to measure, in man, possible release of CCK after oral administration of magnesium sulfate (MgSO₄), and to correlate possible changes in concentrations of plasma CCK (as measured by radioimmunoassay) with gallbladder contraction (as measured by ultrasonography).

Materials and Methods

Five healthy male volunteers from the group of investigators (ages 29-35 years, weight 63-78 kg) participated in this study. The protocol was approved by the Institutional Review Board of The University of Texas Medical Branch, and informed consent was obtained from each individual. Studies were done after a 12-hour fast.

Anteroposterior and transverse echotomograms of the gallbladder were obtained with a Varian phased-array, real-time scan device (Model V-3000) with a 3.5 MHz transducer. To avoid blockage of sound transmission by gas in the colon, the gallbladder was visualized through an intercostal space and the right lobe of the liver. The transducer was maintained at a constant position and angulation to the skin throughout the study of each participant. Assuming that the gallbladder approximates an elliptical cylinder, an approximate volume was determined for each gallbladder using its length, width, and height,¹¹⁻¹⁴ even though accurate measurement of the length of the gallbladder is often impossible. Although the length of the gallbladder usually decreases somewhat during contraction measured by oral cholecystography, it is not always possible to measure this shortening reliably by ultrasonography. The calculated volume, therefore, is an estimate based on the assumption that length is constant. This method provided results that were highly reproducible.

After basal blood samples were drawn and baseline sonograms were obtained, each volunteer drank a so-

lution of magnesium sulfate (25 g in 100 ml of distilled water). Echotomograms were obtained at 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60 minutes after oral ingestion of MgSO₄. Peripheral blood samples were collected at the same intervals. Blood samples for plasma were collected in tubes containing 100 U of Trasylol® (aprotinin) and 10 U of heparin per milliliter of blood. Blood samples were centrifuged at 3000 rpm at 4 C for 15 minutes; plasma and serum samples were stored at -20 C for future measurement of CCK (plasma) and gastrin (serum), and magnesium (serum).

Gastrin was measured by a double-antibody method that has been described previously.¹⁵ Magnesium was measured in serum by atomic absorption spectrophotometry.

Radioimmunoassay of CCK

CCK was measured by a specific radioimmunoassay developed in this laboratory and described in detail previously.¹⁶⁻¹⁸ The CCK antibody (UT 132) was raised in New Zealand white rabbits by repeated inoculation with 16% pure CCK over a 6-month period, followed by one injection of 50 µg 99% pure CCK. The CCK variant (CCK-39), supplied by Professor V. Mutt (Gastrointestinal Hormone Laboratory, Karolinska Institutet, Stockholm), was labeled with ¹²⁵I by the modified Chloramine-T method.¹⁹ The labeled hormone was separated from contaminants on a Sephadex G-10 fine column (0.8 × 16 cm) and further purified on a cellulose CF-11 column (Whatman Chemical Separation, Clifton, NJ) immediately before being used in the assay.

Assay tubes were prepared with 200 µl of plasma, 300 µl of 0.2 M sodium phosphate buffer (PBS, pH 6.8) containing 0.5% normal rabbit serum, 0.15 M sodium chloride, and 0.1% sodium azide, 100 µl antibody solution (final dilution 1:100,000), 100 µl aprotinin solution (four parts PBS, one part aprotinin [10,000 KIU/ml]), and 100 µl disodium ethylenediamine tetraacetate (0.1 M, pH 6.8). The assay tubes were then incubated for 24 hours at 4 C. CCK (200 µl of ¹²⁵I-labeled, 4500 cpm) was then added to each tube and incubated for an additional 48 hours. Bound hormone was separated from free hormone by the double antibody technique. The second antibody, goat antirabbit gamma globulin (100 µl), was added and incubated overnight at 4 C. The assay was terminated by centrifugation at 3000 rpm for 30 minutes, followed by aspiration of the supernatant. The assay tubes containing the pellets were counted for two minutes in a gamma counter.

Concentrations of CCK were calculated using log/logit transformation and expressed in pg/ml. Pure CCK-33, supplied by Professor V. Mutt, was used as the standard. The CCK antibody bound 25%–35% of labeled

CCK-39 at a final dilution of 1:100,000; more than 85% of the tracer was bound with excess antibody. Graded doses of CCK-33, CCK-39, fragments of CCK-33, and gastrin (G-17 and G-34) were measured in the assay system.¹⁸ CCK-33 and CCK-39 were almost equally potent, whereas CCK fragments and gastrin varied in potency from 0.3% to 0.5% when compared with CCK-33 and CCK-39. The same amount of CCK-33 and CCK-39 (5 pg) inhibited binding by 6% and could be detected within the assays with 95% confidence. The intra-assay coefficients of variation for three plasma pools, containing 38, 107, and 248 pg/ml, were 8.7%, 5.5%, and 8.5%, respectively. The interassay variations ranged from 18.5% to 23.2% in five different assays. All samples were measured in duplicate in the same radioimmunoassay.

Statistical Analysis

All values were expressed as the mean ± one standard error of the mean. Student's t-test for paired samples was used to analyze the data. Differences with a p value of less than 0.05 were considered to be significant. Linear regression analysis was performed by the method of least squares. The standard error of estimate (E) in predicting y from a knowledge of x is given for each linear regression line.

Results

Plasma CCK concentrations were increased significantly in response to oral magnesium sulfate. Basal plasma CCK concentrations of 82.2 ± 10.1 pg/ml did not change significantly during the first 15 minutes after ingestion. CCK then rose significantly to 113.8 ± 7.1 pg/ml at 20 minutes, 136.2 ± 10.8 pg/ml at 25 minutes, 144.6 ± 12.6 pg/ml at 35 minutes, and 140.2 ± 17.8 pg/ml at 40 minutes after oral administration of MgSO₄ (Fig. 1).

The basal volume of the gallbladder before administration of magnesium sulfate was 30.8 ± 5.3 cm³. Gallbladder volume did not change in the first 15 minutes; it then decreased as CCK concentrations rose. At 25 minutes, the gallbladder size was decreased significantly to 20.4 ± 3.6 cm³ (Fig. 1). Gallbladder contraction was sustained during the remainder of the study, and the smallest gallbladder volume was observed at 50 minutes (10.9 ± 3.3 cm³) (Fig. 1).

Linear regression analysis demonstrated a significant correlation between plasma CCK levels and gallbladder contraction in response to magnesium sulfate ($r = -0.9337$, $p < 0.01$, $E = 2.82$) (Fig. 2).

Magnesium sulfate also induced a significant increase in serum gastrin levels. Basal serum gastrin levels of 51.4

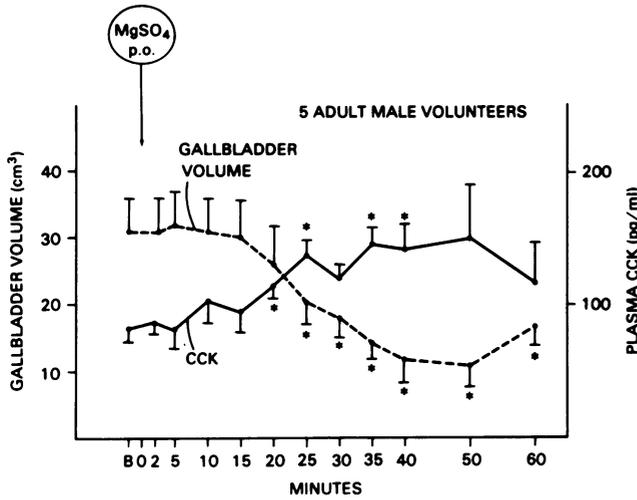


FIG. 1. Changes in concentrations of plasma CCK and gallbladder volume in response to oral magnesium sulfate. * = significant difference from basal.

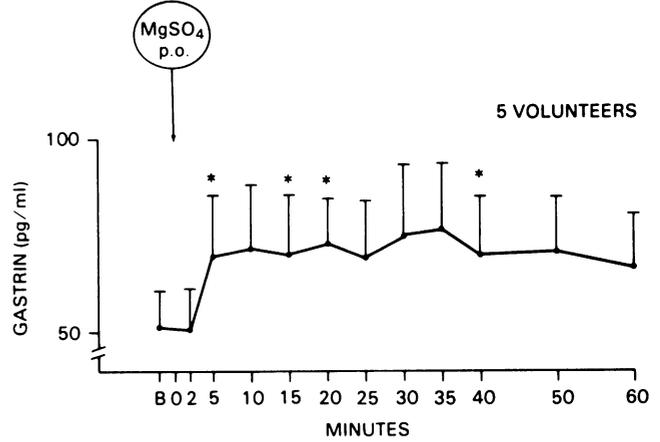


FIG. 3. Changes in concentrations of serum gastrin after oral administration of magnesium sulfate. * = significant elevation above basal.

± 9.9 pg/ml rose significantly to 69.8 ± 15.5 pg/ml at 5 minutes, to 69.8 ± 15.2 pg/ml at 15 minutes, to 62.6 ± 12.1 pg/ml at 20 minutes, and to 69.6 ± 15.3 pg/ml at 40 minutes after oral administration of magnesium sulfate (Fig. 3). Linear regression analysis showed no significant correlation between serum gastrin levels and gallbladder contraction ($r = -0.5124$).

There was no significant change in serum magnesium concentrations from a mean basal concentration of 3.1 ± 0.2 mg/dl.

All the volunteers noted hyperperistalsis and had increased bowel movements—an average of three—from 30 minutes to 24 hours after oral ingestion of magnesium sulfate.

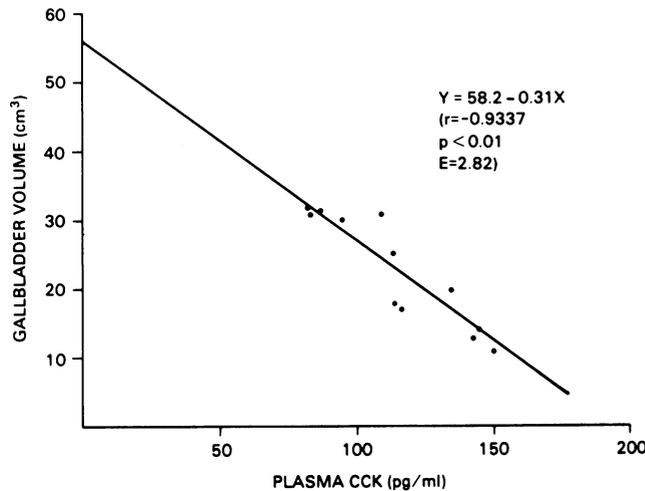


FIG. 2. Linear regression analysis of CCK levels and gallbladder volume during 60 minutes after oral administration of magnesium sulfate.

Discussion

The stimulatory effect of magnesium sulfate on gallbladder contraction has been demonstrated by measuring bile flow²⁻⁷ or by cholecystography.^{3-5,8,9} MgSO₄ also relaxes the sphincter of Oddi.^{5,20} In 1943, Boyden and colleagues⁵ reported that magnesium sulfate acts to produce gallbladder emptying for the same length of time as egg yolk and differs merely in the degree to which it affects the gallbladder contraction or the relaxation of the sphincter of Oddi. They suggested that the mechanism is not local but humoral, probably mediated by the release of CCK-like substance. Malagelada and colleagues^{6,7} have provided bioassay data that suggests that magnesium sulfate causes release of CCK from the duodenum, and there is a brief report without experimental data by Harvey and colleagues²¹ that a rise in CCK was measured by radioimmunoassay after oral administration of MgSO₄. The authors' study confirms that oral administration of magnesium sulfate stimulates a significant increase in plasma CCK levels measured by specific radioimmunoassay.

The introduction of the Graham-Cole method of cholecystography^{22,23} made it possible to measure the reduction in size of gallbladder at time intervals in response to choloretic agents such as magnesium sulfate. Controversy exists as to the time of maximal gallbladder contraction (measured by cholecystography) with a range of 33,⁸ 35,⁵ 40,⁴ and 55 minutes³ after the intraduodenal instillation of magnesium sulfate. The insensitivity of cholecystographic techniques may explain some of the discrepancies. Braverman and colleagues¹² showed that ultrasonography is an acceptable way of assessing the approximate gallbladder volume. This present study corroborates that finding.

A significant increase in CCK concentrations occurred more rapidly (20 min) than did a significant re-

duction of gallbladder size (25 min) in response to oral magnesium sulfate. This is similar to the findings with fat,¹³ except that after oral fat, significant release of CCK was found at 15 minutes, with a similar lag period before significant gallbladder contraction. The relationship between initial release of CCK and initial gallbladder contraction in this study and in the previous study with fat¹³ provides strong evidence that active forms of CCK are measured.

Simultaneous measurement of gastrin in this study showed a significant increase in serum gastrin levels in response to oral magnesium sulfate. It has been shown previously that magnesium sulfate given intravenously releases gastrin in patients with the Zollinger–Ellison syndrome,²⁴ and Christiansen and colleagues²⁵ reported that addition of magnesium sulfate to the intravenous infusion of calcium gluconate caused a slight, but not significant increase in serum gastrin concentration in normal man. To the authors' knowledge, no prior information is available to indicate the effect of oral or intraduodenal magnesium sulfate on gastrin release. This study showed a rapid increase of serum gastrin levels after oral magnesium sulfate. High concentrations of magnesium sulfate presumably stimulate gastrin release from the antral mucosa. The physiologic role of this phenomenon is not clear.

In conclusion, CCK, as measured by a specific radioimmunoassay, is released by oral magnesium sulfate in man. Gallbladder contraction is closely correlated with measured concentrations of CCK in response to magnesium sulfate. This study provides direct evidence that magnesium sulfate stimulates the gallbladder to contract by releasing CCK.

Acknowledgement

We would like to thank Ms. Freddie L. C. Hill, Mr. Danny McKay, Ms. Julie Gips, and Mrs. Marilyn A. Thompson for their expert assistance in the preparation of this study.

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