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Effect of Intravenous C-Terminal Octapeptide of Cholecystokinin and Intraduodenal Ricinoleic Acid on Contractile Activity of the Dog Intestine (39363)

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Contractions of the circular smooth muscle of the gastrointestinal (GI) tract are reduced substantially in the unanesthetized dog after oral cathartic doses of castor oil (1). In order to study further the source of the smooth muscle inhibition, we conducted a series of experiments in which the active ingredient of castor oil, ricinoleic acid (12-hydroxyoleic acid), was infused directly into the duodenum of dogs previously fed a meat meal (2). After a brief (<2 min), initial stimulation, the fatty acid produced a prolonged (20 min) inhibition of food-induced contractile activity over a considerable portion of the GI tract. Ricinoleic acid, therefore, infused into the lumen of the proximal small intestine altered muscle contractions in areas of the GI tract removed from the infusion site. This suggested that the effects of castor oil and ricinoleic acid on GI smooth muscle *in vivo* were mediated indirectly, possibly by a duodenal hormone.

This study was conducted to compare the changes in ongoing digestive contractile activity after intraduodenal (ID) administration of ricinoleic acid and intravenous injection of the C-terminal octapeptide of cholecystokinin (CCK-OP). We have found that both procedures produce similar changes in the digestive contractile patterns of the small intestine and that the initial stimulatory response after both procedures is mediated through a cholinergic mechanism.

Materials and methods. Extraluminal strain gage transducers were implanted in two male, mixed breed dogs weighing 10 and 12 kg. The procedure was performed under aseptic surgical conditions with the animals anesthetized with sodium pentobarbital (30 mg/kg iv). A detailed account of transducer construction and implantation has been published (3). The units were sewn onto the serosal surface of the organ to be studied along the transverse axis to monitor the contractile activity of the circular smooth muscle. Eight transducers were implanted in each animal and positioned on the proximal jejunum (6 and 12 cm caudad to the ligament of Treitz), on the middle jejunum (60 and 66 cm caudad to the ligament of Treitz), on the terminal ileum (14 and 20 cm orad to the ileocecal valve), and on the proximal colon (6 and 12 cm caudad to the ileocecal valve). In addition, each animal was implanted with a duodenal cannula placed approximately 8 cm distal to the gastroduodenal junction. The contractile activity of the circular smooth muscle was recorded by a Beckman Dynograph (Type R411).

The animals were housed in a constant temperature environment and were maintained on a 12-hr light-dark cycle. They had access to water *ad libitum* and received a daily ration of dry dog food. Experiments were performed on the unanesthetized animals no sooner than 10 days after surgery. Experiments were begun between 1 and 3 PM, and the animals lay quietly in a limited access laboratory for the duration of each experiment.

One-half hour before each experiment the animal was fed 200 g of canned dog food to induce continuous contractile activity in the areas monitored. Alterations in digestive contractile patterns were studied after the following procedures: 1) iv bolus injection of CCK-OP, 50 or 500 ng/kg; 2) repeated iv bolus injections of CCK-OP (50 ng/kg) spaced at 5-min intervals; 3) multiple ID bolus infusions of sodium ricinoleate (500 mg, Pfaltz-Bauer, Inc.) at 5-min intervals; 4) the action of atropine sulfate (100 μg/kg sc) on the motor effects produced by iv CCK-OP or ID ricinoleic acid; 5) the action of hexamethonium (10 mg/kg sc) on the motor effects of ID ricinoleic acid. For

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procedure 3, the sodium salt of ricinoleic acid was dissolved in 30 ml of isotonic saline and infused into the duodenal cannula over a 2-min period. A 30-ml volume of isotonic saline adjusted to the pH of the fatty acid solution (9.5) served as control. Each procedure was repeated at least twice in each animal.

Results. CCK-OP produced a biphasic response when given as an iv bolus (Fig. 1). At 50 ng/kg, there was an initial stimulation of digestive contractile activity in the proximal and middle jejunum followed by a post-stimulatory inhibition (PSI). The digestive patterns of the terminal ileum and proximal colon were unaffected. A tenfold increase in dose (500 ng/kg) produced a greater initial stimulation followed by a longer PSI. In contrast to the lower dose, the higher dose affected all areas (Fig. 1). The higher dose also caused the animals to lose consciousness momentarily, apparently due to a fall in blood pressure.

Repeated iv bolus injections of CCK-OP always induced the characteristic response (Fig. 2, upper tracings) of initial stimulation followed by post-stimulatory inhibition. ID bolus infusion of ricinoleic acid affected digestive activity in exactly the same manner. However, repeated ID infusions of the fatty acid produced increasingly diminished stimulatory responses (Fig. 2, bottom tracings). The reduced responses following the second and third ID infusion of ricinoleic acid occurred after an increased time period. ID infusion of control saline did not affect digestive contractile activity.

Atropine completely antagonized the motor effects of iv CCK-OP and ID ricinoleic acid (Fig. 3, upper tracings); hexamethonium only partially antagonized the effects of ID ricinoleic acid (Fig. 3, bottom tracings).

Discussion. The gastrointestinal hormone, cholecystokinin, is released into the peripheral circulation by the presence of fatty acids or amino acids in the lumen of the small intestine (4, 5). Both the hormone and its C-terminal octapeptide have been found to stimulate the contractile activity of the small intestine and colonic smooth muscle (6–9). These effects have been confirmed in the present study. However, we make three additional observations regarding the motor actions of CCK-OP administered by rapid iv bolus: 1) the proximal and

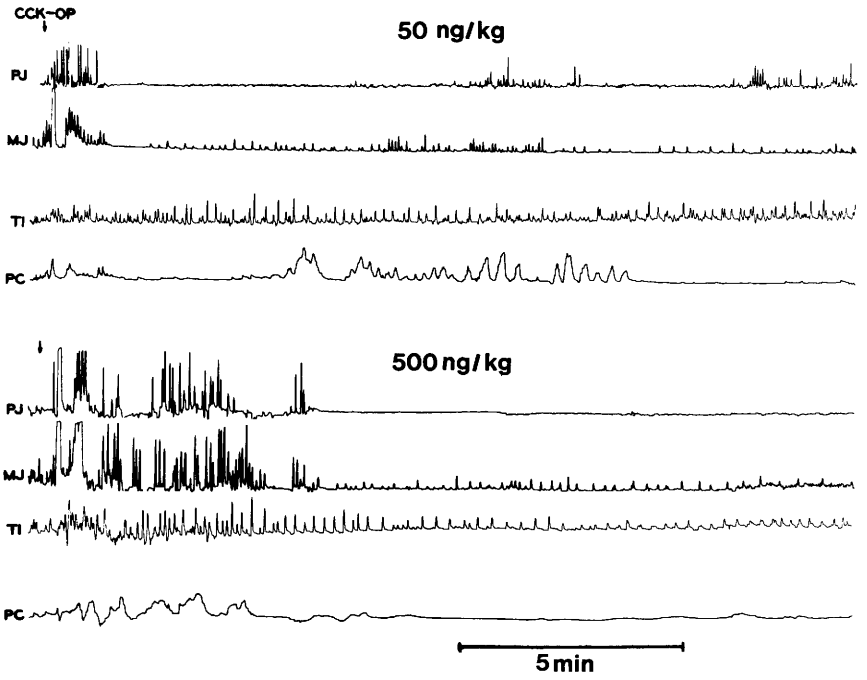

FIG. 1. The effects of iv bolus injection of CCK-OP (50 and 500 ng/kg) on the digestive contractile activity of the proximal (PJ) and middle jejunum (MJ), terminal ileum (TI), and proximal colon (PC).

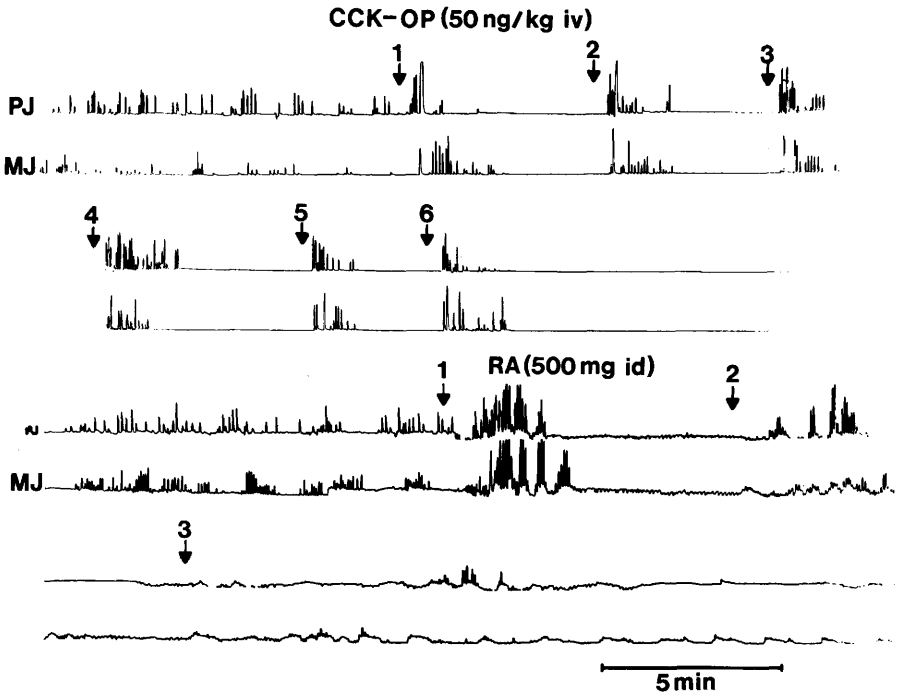

FIG. 2. The top tracings show the effects of six successive iv bolus injections of CCK-OP on the digestive contractile activity of the proximal (PJ) and middle jejunum (MJ). The bottom tracings show the response after the first and the diminished stimulatory responses following the second and third intraduodenal (id) bolus infusions (2-min duration) of ricinoleic acid (RA). Note the greater time period required for the responses to the second and third intraduodenal bolus of RA.

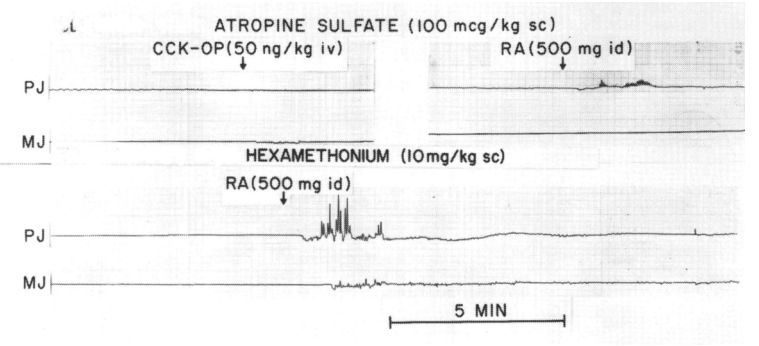

FIG. 3. The action of atropine sulfate on the effects of iv CCK-OP and intraduodenal ricinoleic acid (RA) on the digestive contractile activity of the proximal (PJ) and middle jejunum (MJ) is shown in the top tracings. The bottom tracings demonstrate the inability of hexamethonium to antagonize the intraduodenal ricinoleic acid response completely.

middle small intestine are affected preferentially by lower doses of the compound; 2) when the effects of iv CCK-OP are superimposed upon an established, physiologic level of contractile activity induced by food, the initial stimulation of motor activity is followed by inhibition; 3) the motor actions of iv CCK-OP showed marked similarities to the motor responses evoked by infusion of ricinoleic acid directly into the duodenum. The latter observation suggests that the motor effects following infusion of a fatty acid into the proximal small intestine may result predominately from release of endogenous cholecystokinin.

The motor response after ID ricinoleic

acid was similar in all but one respect to the motor effects of iv CCK-OP. Unlike repeated iv administrations of the octapeptide, successive challenges with ID fatty acid failed to produce continued responses. If the stimulatory motor actions of ID ricinoleic acid do result from the release of endogenous cholecystokinin, then feeding a meat meal and the subsequent presence of the contents in the small intestine must not release all stores of the hormone completely. It may require repeated infusion of a potent releaser of cholecystokinin, such as a fatty acid, directly into the small intestine to exhaust this residual store of hormone. The greater time period required for the diminished responses to the second and third ID bolus of fatty acid might result from the release of more distal stores of cholecystokinin. This is compatible with the observation that releasable cholecystokinin extends beyond the most proximal small intestine and decreases with increasing distance from the pylorus (10). Alternately, the failure of continued ID infusions of ricinoleic acid to produce a response might result from a desensitization of the hormone release mechanism.

The stimulant effect of cholecystokinin on GI smooth muscle appears to be cholinergically mediated. Recent investigations present evidence that CCK interacts with non-nicotinic receptors on the ganglion cells of Auerbach's plexus to release acetylcholine at neuroeffector junctions (11–13). The present investigation supports these findings since atropine antagonized the stimulatory response to iv CCK-OP. Furthermore, atropine, but not hexamethonium antagonized the motor effects of ID ricinoleic acid. Thus the stimulatory effects of both ID ricinoleic acid and iv CCK-OP are mediated by a cholinergic mechanism.

The source of the PSI following iv CCK-OP or ID ricinoleic acid noted in this study is unknown. The fact that the response was obtainable after both procedures suggests that its source is a high circulating level of cholecystokinin. However, the PSI response may not be a direct action of the hormone. Glucagon, a hormone known to inhibit motor activity of the small intestine (14, 15) is released into the circulation during high circulating levels of cholecystokinin (16, 17) and may be the ultimate source of the response.

Equal quantities of oleic acid and ricinoleic acid infused into the proximal small intestine produce similar alterations in digestive contractile patterns. Quantitatively, however, the inhibitory phase of the response to ricinoleic acid is much greater (2). Similar differences are noted after oral administration of the fatty acids. Oral oleic acid (10 g) alters digestive contractile patterns briefly. Oral ricinoleic acid (10 g) produces a prolonged (4 to 6 hr) inhibition of digestive contractile patterns after which the animal produces a wet stool (2). These observations indicate that the major difference in the motor patterns induced by a diarrheogenic and nondiarrheogenic fatty acid, either orally or intraduodenally administered, is the greater ability of the diarrheogenic fatty acid to maintain the period of circular smooth muscle inhibition. Whether this difference in turn reflects differences in hormone releasing ability between the fatty acids remains to be determined.

Summary. Intraduodenal infusion of ricinoleic acid and iv bolus injection of the C-terminal octapeptide of cholecystokinin produce markedly similar alterations in the digestive contractile patterns of the GI tract of the unanesthetized dog. The brief, initial stimulation of contractile activity in the proximal small intestine following both procedures is mediated through a cholinergic mechanism. The stimulatory response is followed by an inhibition of digestive contractile activity of unknown origin. These observations suggest the possibility that the GI hormone, cholecystokinin, may mediate the intestinal motor response evoked by infusion of ricinoleic acid directly into the proximal small intestine.

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