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ROLE OF CHOLECYSTOKININ IN POSTPRANDIAL AND VAGALLY STIMULATED DUODENAL AND GALLBLADDER MOTILITY IN DOGS

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This study was designed to determine the role of cholecystokinin (CCK) in postprandial motility pattern of the duodenum and gallbladder (GB) in conscious dogs provided with chronic duodenal electrodes for recording of myoelectric activity and GB fistulas for measurement of intraluminal pressure and volume of GB and to calculate the GB motility index (MI) and GB emptying rate. During naturally occuring activity front (phase III MMC) in the duodenum there was significant increase in the MI of GB accompanied by about 20—30% reduction in the GB volume. These changes in duodenal and GB motility pattern could be duplicated by i. v. motilin. Feeding abolished the appearence of spontaneous activity front in the duodenum and greatly increased motility of GB while reducing its volume. Administration of CCK receptor antagonists in fed dogs failed to affect the motility changes induced by meal in the duodenum but abolished these of the GB. Vagal cholinergic stimulation with insulin, 2DG or urecholine caused similar effects to that induced by food i. e. increased duodenal spike activity, abolished phase III of the MMC, decreased GB volume and increased GB motility. Pretreatment with CCK antagonists did not affect significantly duodenal spike activity or GB motility but significantly increased the GB volume. Atropine 125 μg/kg) blocked almost completely spontaneous activity front in the duodenum and accompanying alterations in the motility and volume of GB. We conclude that CCK contributes to the MMC related alterations in the GB motor activity and is essential in cholinergic stimulation induced of the GB emtying but not in vagally induced duodenal and GB motility.

Key words: *gallbladder, motility, vagal stimulation cholecystokinin, fed pattern.*

INTRODUCTION

Since Ivy and Oldberg (1) discovered cholecystokinin (CCK) in 1928 numerous studies provided evidence that this hormone plays a crucial role in the GB contractions and emptying after feeding (2—4). Recent studies using highly sensitive CCK radioimmunoassay and specific CCK receptors antago-

nists provided further support for the dependence of the postprandial GB contractions upon the release of CCK (5) but little information is available regarding the role of CCK in GB contraction in response to ordinary feeding and vagal cholinergic stimulation.

The present study was designed to clarify the role of CCK in duodenal and GB motility pattern following meat feeding and vagal cholinergic stimulation such as induced by 2-deoxy-D-glucose (2DG), insulin and urecholine using the most potent CCK receptor antagonist (L-364, 718) in dogs provided with chronic duodenal electrodes for simultaneous determination of the myoelectric activity and with the GB fistulas (GBF) for measurement of GB intraluminal pressure and emptying rate.

MATERIAL AND METHODS

Experiments were carried out on 10 mongrel dogs (15—20 kg b. wt.) prepared surgically with intestinal electrodes as decribed before (4) and with the GB fistulas. Three monopolar electrodes were implanted in duodenum, for recording of myoelectric activity. The fistulas of the GB were prepared by inserting 20 cm long flanged stainless steel cannula (5 mm inner diameter) into the GB via a small incision in its fundus. The cannula was firmly anchored to the GB wall using purse string suture. The other end of the cannula was brought out through a stab wound in the right upper abdominal wall and closed by a cork to prevent bile loss. Experiments were carried out at least 3 wk after surgical procedure. After an 18 h fast, with free access to water, each dog was placed on the Pavlov stand. Recordings of duodenal myoelectric activity were made with a type R-611 Beckman recorder with the couplers of 9658A as described before (2). The GB motility and volume (or emptying) were measured using, respectively, Statham pressure transducer (Howell, USA) to record continuously the intraluminal pressure and a plastic syringe attached to the GB cannula to measure periodically the GB volume. The biliary catheter was constantly perfused with saline at a constant rate of 0,05 ml min. The calibration of the pressure recording system was tested by elevating the tip of saline-filled biliary catheter to various levels above the entry GB cannula in the abdominal wall that was considered as a zero level. Motility index (MI) was calculated from the sum of pressure amplitudes obtained by counting all elevations in the pressure of 2 or more cm H_2O value above the basal zero line multiplied by the number of contractions in each of 2 min periods.

Several series of tests were performed under basal conditions and following ordinary feeding or pharmacological vagal cholinergic stimulation (insulin, 2DG, urecholine) and CCK8 without or with addition of CCK receptor antagonist. At least two control MMC cycles were first examined and accompanied changes in the GB volume and motility index were recorded in each 15 min period. Intravenous infusion of saline was given at a rate of 40 ml/h from the start of all experiments and during of the control MMC cycles. CCK-receptor antagonist L-364, 718 (gift of Dr P. S. Anderson, Merck, Skarp & Dohme, West Point, PA) was added to i. v. infusion in single bolus dose. The duodenum and GB motor activity were measured for the next 2—3 h. In test with feeding 500 g of cooked homogenized ground beef was offered for 5 min period.

Pharmacological vagal cholinergic stimulation was achieved by administration of insulin (0.2 U/kg-h), 2DG (50 mg/kg-h) or urecholine 50 μg/kg-h) given as i. v. infucion. After about well sustained plateau, L-364, 718 was administered i. v. in a single bolus injection of 0.5 mg/kg. Infusion of insulin, 2DG or urecholine was continued for the next 2 h. For comparison, CCK8

was infused at a constant dose of 100 ng/kg-h with or without addition of L-364, 718 in a bolus dose of 0.5 mg/kg.

Results are expressed as a means ±SEM. The significance of the difference between means was evaluated by analysis of variance followed by Student's "t" test. Differences were considered significant if $p < 0.05$.

RESULTS

Fig. 1 shows the periodic changes in the myoelectric activity of duodenum with typical activity front or phase III of MMC accompanied by the increase in the motor activity of the GB and reduction in its volume. Meat feeding interrupted the periodic myeolectric activity of the duodenum resulting in a continuous spike activity with about 30% slow waves with spikes. The motility of the GB greatly increased and its volume was reduced by about 85% *Fig. 1.*

Following i. v. administration of L-364, 718 (0.5 mg/kg), no alterations in periodic myoelectric activity of duodenum were observed but, as reported previously (2), the MMC interval increased significantly to about 126±12 min *Fig. 2.* There was also significantly smaller increase in the MI of the GB during phase II/III in duodenum and the GB volume was not reduced during phase II/III of MMC in duodenum. Motilin given i. v. as a single bolus injection of 80 ng/kg in L-364, 718 pretreated dogs resulted in a typical activity front in duodenum accompanied by usual increase in GB motility index and reduction in the GB volume. L-364, 718 given i. v. in fed dogs did not affects the myoelectric activity of duodenum but abolished GB motility and volume

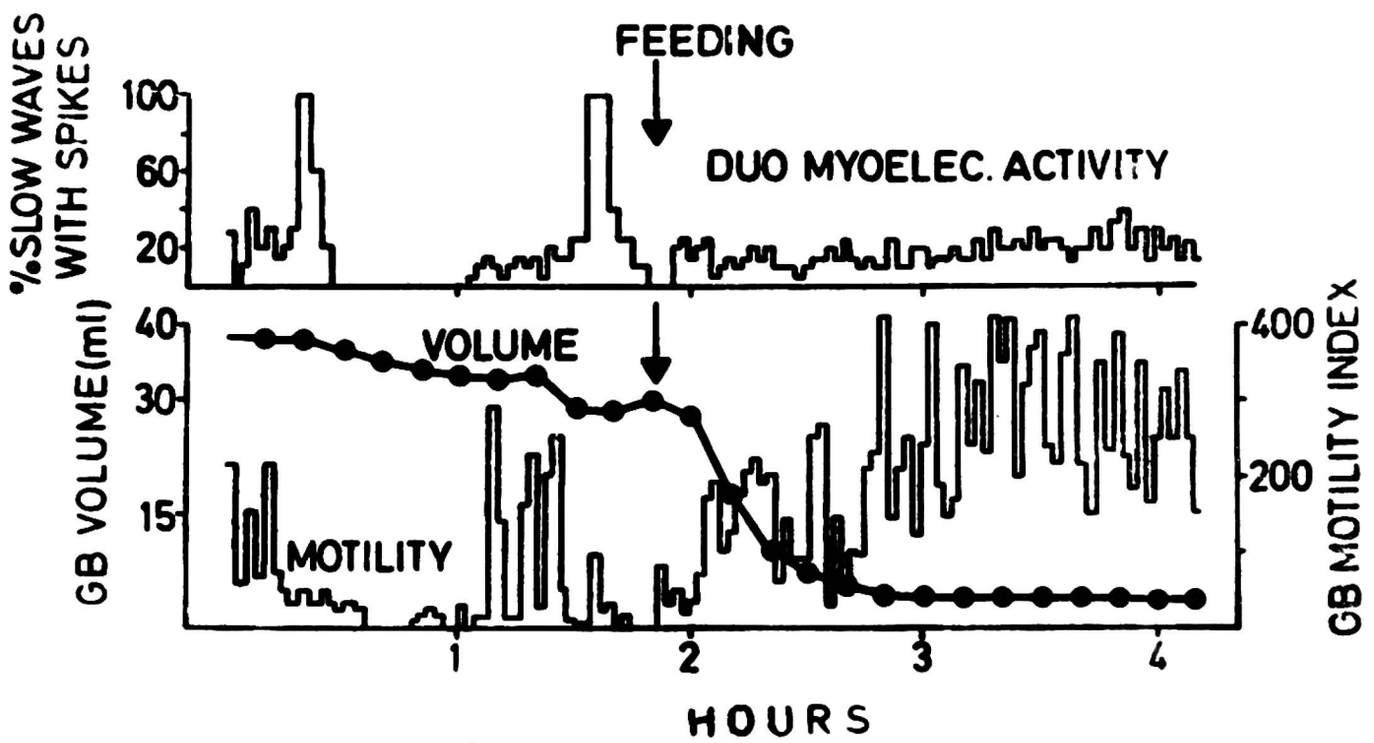

Fig. 1. Effect of feeding (500 g meat) on myoelectric activity of duodenum and GB volume and motility. Similar results were obtained in all dogs.

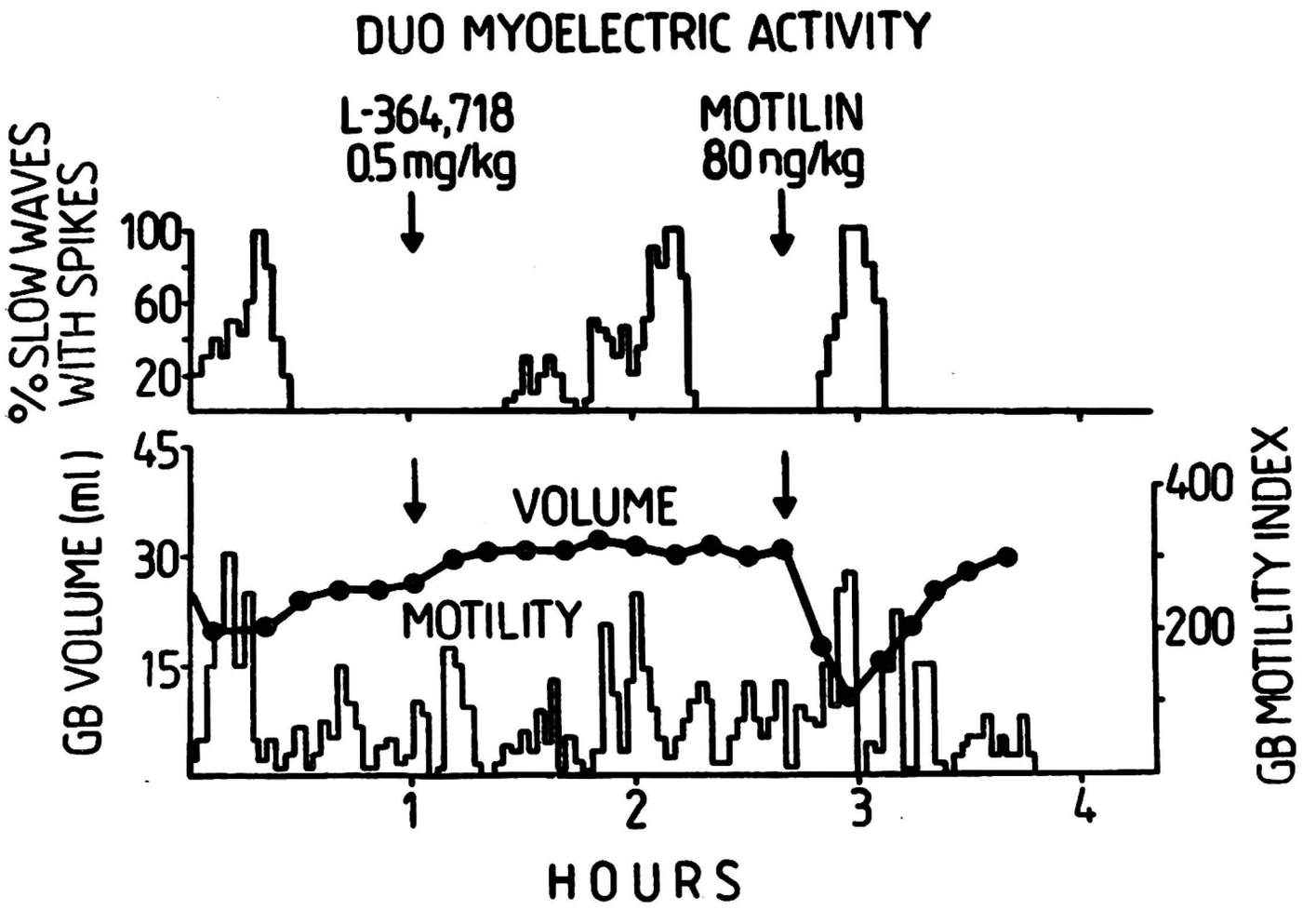

Fig. 2. Effect of CCK receptors antagonist L-364, 718 given i. v. in bolus dose of 0.5 mg kg on duodenal myoelectric activity and GB volume and motility in fasted dogs. Similar results were obtained in all dogs.

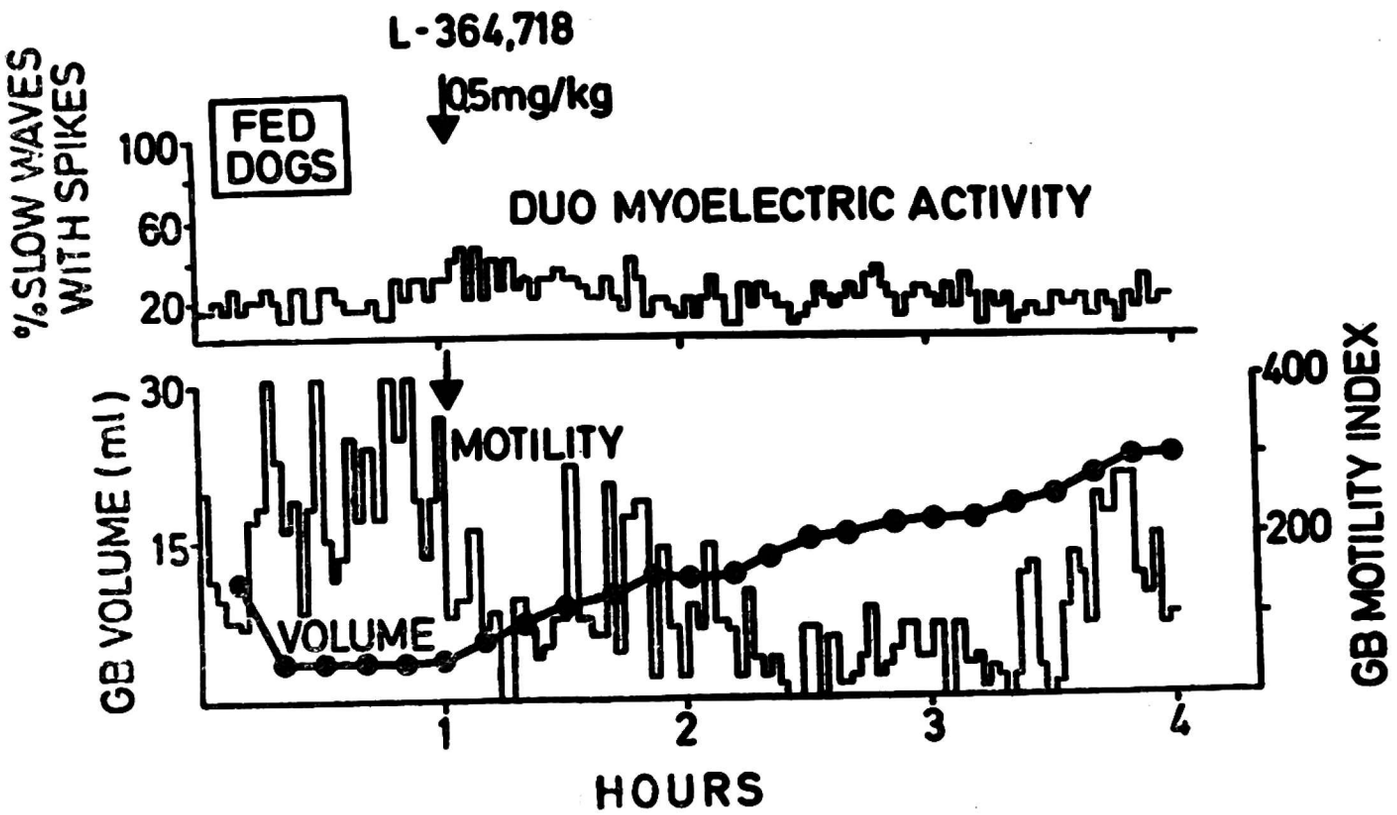

Fig. 3. Effect of CCK receptors antagonist L-364, 718 given i. v. in fed dogs on duodenal myoelectric activity and GB volume and motility. Similar results were obtained in all animals.

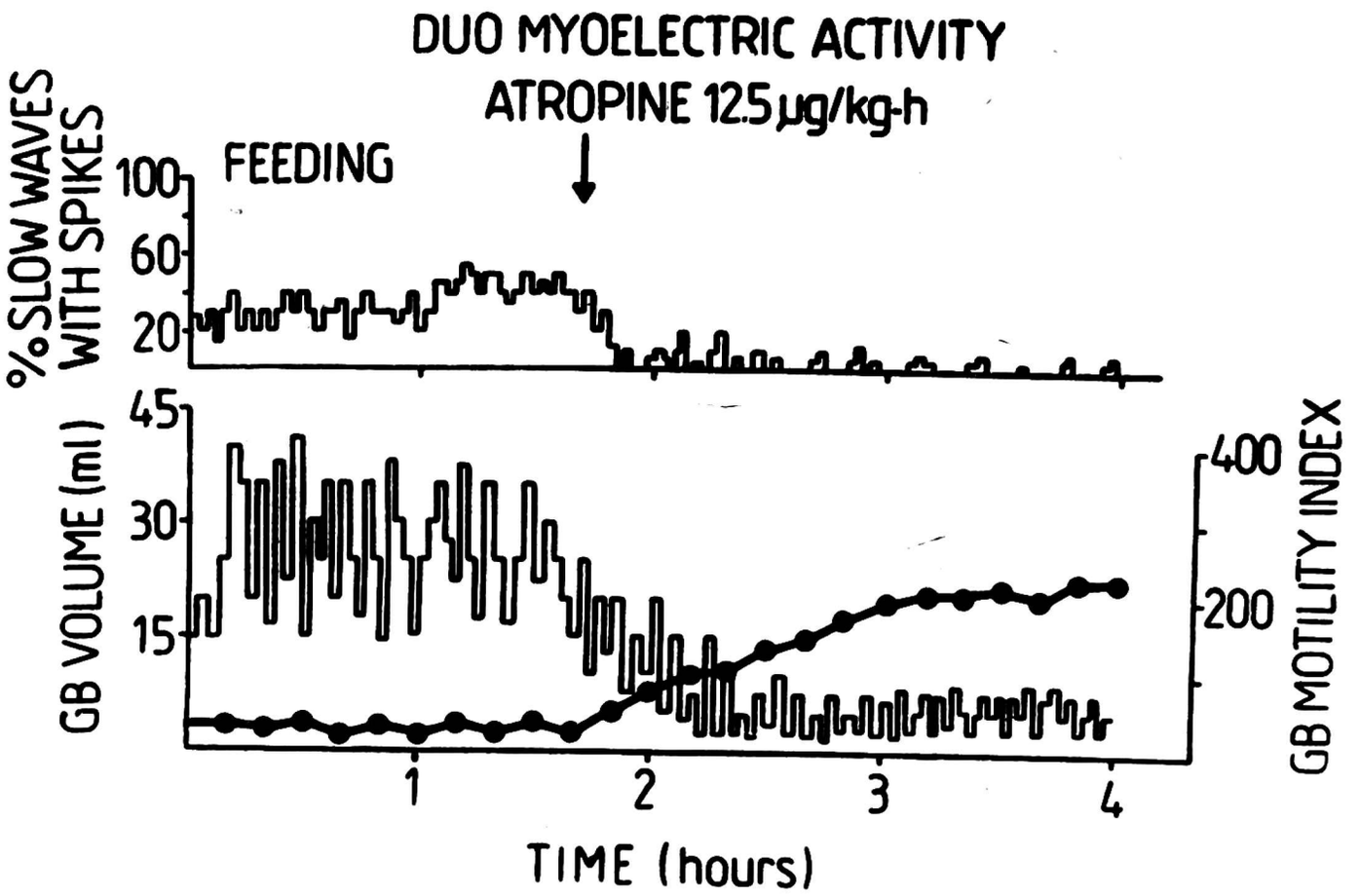

Fig. 4. Effect of atropine given in i. v. infusion in a dose of 12.5 µg kg-h in fed dogs on duodenal myoelectric activity and GB volume and motility. Similar results were obtained in all animals.

changes induced by meal. On the contrary, the GB volume increased and MI decreased during the first hours after administration of CCK antagonist *Fig. 3.* Atropine (12.5 µg/kg) given i. v. in bolus dose in fasted dogs abolished periodic myoelectric activity of the duodenum, reduced the and increased GB volume (Data not shown). Atropine administered in fed dogs also abolished almost completely activity of the duodenum while reducing the GB contractility and increasing GB volume *Fig. 4.* These effects of atropine on the GB motility and volume were similar to that observed after L-364, 718. Vagal cholinergic stimulation with insulin, 2DG or urecholine caused similar effects to that induced by food namely it increased duodenal spike activity and abolished phase III in the duodenum MMC, decreased significantly GB volume and increased GB MI. Blockade of CCK receptors by L-364, 718 in dogs infused with with insulin, 2DG, urecholine did not affect significantly duodenal spike activity or the GB motility but significantly increased the GB volume *Fig. 5.*

DISCUSSION

The GB performs two distinct but physiologically related functions, absorbs water and electrolytes and contracts to deliver its contens into the duodenum.

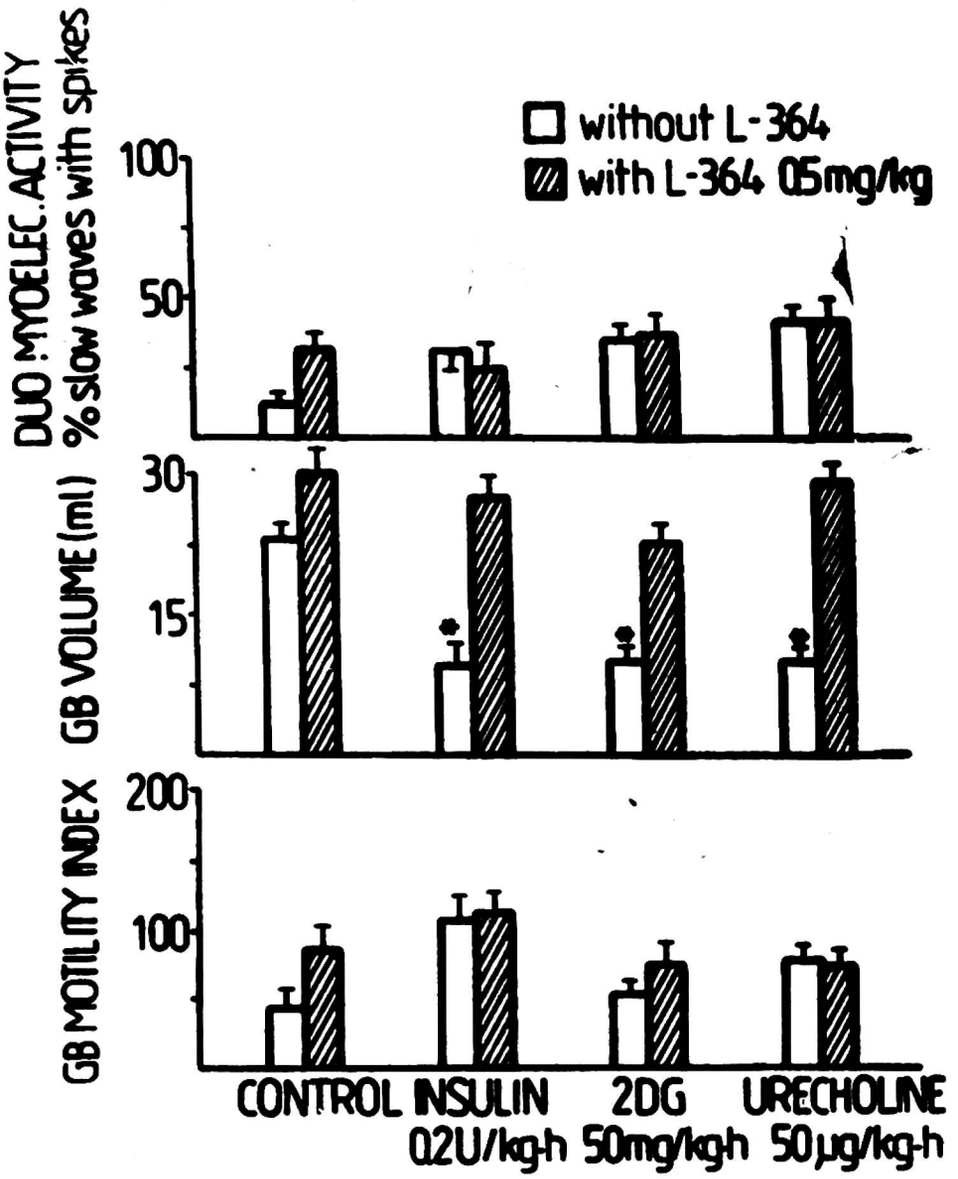

Fig. 5. Effect of vagal stimulation with insuline (0.2 U kg-h), 2DG (50 mg kg-h) and urecholine (50 ug kg-h) with and without L-364, 718 on duodenal myoelectric activity and GB volume and motility in fasted dogs. Each point is mean ± SEM of tests on 10 dogs. Asterisk indicate significant decrease in GB volume.

This study provides evidence that postprandial changes in the motor activity of the duodenum and GB occuring after feeding involve both the cholinergic component and CCK. As has been shown previously (4) exogenous and endogenously released CCK is responsible, at least in part, for interruption the cyclic interdigestive MMC activity of the small bowel and for induction of continuous spike activity, changes similar to that induced by ordinary feeding. Available evidence suggests (6) that CCK is one of the main factors responsible for inducing strong contraction of the GB and the GB emptying by mediating both the potent contraction of the GB and concurrent relaxation of the sphincter Oddi (SO).

Most previous studies investigating the action of CCK on the GB concentrated on the contractions or emptying of the GB. Wiener and Thomson (5) were first to describe a close correlation between plasma CCK concentration and changes in GB volume. In dogs, Fried et al (7) observed that intraduodenal

infusion of sodium oleate resulted in increased plasma CCK concentration that paralleled increase in the intraluminal GB pressure.

Despite the obvious effects of acetylocholine and vagal stimulation on the GB motility it has been shown that increased pressures were not associated with evacuation of the GB (8). Thus the physiological significance of the vagal innervation in the GB functions remains uncertain. Truncal vagotomy resulted in a significant diminution of fat stimulated GB contractions, although no difference was found in CCK release after vagotomy (9). A number of previous reports indicated that GB tone and motility were under vagal influence (10—12). Johnson and Boyden (9) reported decreased emptying after vagotomy. It appears, therefore that vagal innervation of the GB is responsible for the tone of the GB whereas emptying of this organ is primarily under hormonal influences.

Studies reported by Niederau and Karaus (13) showed that CCK antagonists decreased contractile activity of the muscles strips from canine ileum and proximal colon. This study showed that combined application of caerulein and acetylocholine caused an increase in intestinal motility which was greater than that induced by either substance separately. In our in vivo experiments vagal cholinergic stimulation with insulin, 2GD or urecholine increased duodenal spike activity and enhanced both the motor activity and emptying of the GB. It induced fed-like pattern of the small bowel by abolishing the MMC cyclic activity. Infusion of CCK antagonists in vagally stimulated dogs did not changed the duodenal spike activity or the GB motor activity but reduced the GB emtying, suggesting the involment of the cholinergic component in the GB emptying. GB emptying depends not only upon its motor motility but also upon the activity of SO. As has been shown by Suzuki and Itoh (14), SO motility is also modulated by neural input and cholinergic nerves may have some role, because cutting and reanastomosing of SO in opossum uncoupled the spike activity in the proximal and distal SO. Thus, increased cholinergic activity raises the motor activity of the SO and this may be partially responsible for increased emptying of GB observed in our experiments after vagal stimulation.

Increased emptying of GB observed after cholinergic stimulation could be also attributed to direct effects of cholinergic stimulation on the GB contractility. There is evidence in the literature of a vago-vagal antrocholecystic reflex. Debas and Yamagishi (15) reported that inflation of a baloon in the antrum of concious dogs induced GB contraction and increase bile output. This reflex was blocked by thoracic vagotomy and atropine. Feeding did not changed normal postprandial response of the SO to surgical and phenol red treatment which suggests that this response is mostly hormonal.

Our results with CCK antagonist support the conclusion that CCK plays a crucial role in motor activity of the GB and in the emptying of this organ.

Similar observations were made by Itoh et al (16) who stated that postprandial response of the SO was antagonized by proglumide. Thus the decrease in GB emptying and motility after blockade of CCK receptors could be caused by actions of those drugs on both GB motility and SO pressures resulting in alteration in the GB amptying. The lack of response of the GB to vagal stimulation after pretreatment with CCK receptors antagonist suggests that hormonal influences can modify the neuronal input to the GB.

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