Nifedipine Inhibits Cholecystokinin Induced Gallbladder Contraction.

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Abstract:

MSc Surgery David Clas Experimental

Nifedipine Inhibits Cholecystokinin-Induced Gallbladder Contraction.

The purpose of this study was to show that nifedipine, a calcium channel blocker, can decrease gallbladder contractility in guinea pigs and in man Gallbladder contraction was measured in response to repeated injections of cholecystokinin both before and after the injection of nifedipine in three groups of five animals each. The mean amplitude of gallbladder contraction in response to cholecystokinin was decreased by 45, 73 and 67 % ($p<0.01$) in response to intravenous nifedipine doses of 100, 200 and 300 µg respectively In nine healthy human volunteers, gallbladder emptying was measured by radionuclide cholescintigraphy in response to cholecystokinin infusion before and after a 10 mg oral dose of nifedipine. Gallbladder ejection fraction was significantly decreased by 29 % ($p<0.001$). These data demonstrate that nifedipine is a potent inhibitor of gallbladder contractility

Resumé:

MSc David Clas Chirurgie experimentale

La nifedipine inhibe la contraction biliaire due à la cholecystokinine.

Le but de cette étude est de démontrer que la nifedipine, un bloqueur des canaux calciques, bloque la contraction biliaire chez les cochons d'Inde et chez l'homme. La contraction biliaire induite par des injections répétées de cholecystokinine avant et aprés l'administration de nifedipine a été mesurée dans trois groupes de cinq cochons d'Inde. L'amplitude moyenne de contraction biliaire causée par la cholecystokinine a été diminuée de 45, 73 et 67 % (p,0.01) suite à des doses de nifedipine de 100, 200 et 300 µg par voie intraveineuse. Chez neuf volontaires humains sains, la contraction biliaire induite par une infusion de cholecystokinine a été mesurée par la cholescintigraphie radionucléaire avant et aprés une dose orale de 10 mg de nifedipine La fraction d'éjection de la vésicule biliaire a été diminuée de 29 % ($p<0.001$). Cette étude démontre que la nifedipine est un puissant inhibiteur de la contraction biliaire.

Preface:

The *in vivo* animal model used in this study was previously developed by Drs Hould, Fried and Mersereau (1) This thesis presents original research making use of this model This study and its results has been published in a peer reviewed journal prior to completion of the current text (2) and thus represents a contribution to scientific knowledge. The experimental design involving animals was approved by the McGill University and The Montreal General Hospital Animal Care Committees The human study was approved by The Montreal General Hospital Clinical Trials Committee

I wish to acknowledge and thank Dr G.M. Fried, Dr W.A. Mersereau and Dr R.C. Chiu for their help and support during the completion of this study.

Introduction:

The importance of calcium fluxes in smooth muscle contraction is well recognized. In contrast to the action potential of skeletal muscle, the action potential of smooth muscle is calcium dependent.

Nifedipine is one of a group of calcium channel blocking drugs that has been used clinically in the management of angina and hypertension. Calcium channel blockers cause their clinical effect by acting on the smooth muscle of the blood vessels. The entire gastrointestinal tract, including the gallbladder, contains smooth muscle. Although the use of nifedipine has been studied in the treatment of a variety of gastrointestinal motility disturbances, its effect on gallbladder motility has not yet been well characterized.

The purpose of this study was to investigate, both in an *in vivo* animal model and in man, the effects of the calcium channel blocker nifedipine on the cholecystokinin-induced contraction of the gallbladder. We hypothesized that nifedipine diminishes gallbladder contractility. This effect may be potentially useful clinically in the management of biliary colic. As motility disorders of the gallbladder are implicated in gallstone formation, any decreased gallbladder motility due to calcium channel blockers used for other therapeutic purposes may cause gallstone disease in the many patients on these drugs.

In man, the gallbladder is a hollow pear shaped appendage of the extrahepatic biliary tree. It is found on the undersurface of the liver in a depression on the right lobe. It is normally from 7 to 10 cm long and 2 to 3 cm wide. The volume of the gallbladder varies but is usually from 35 to 50 ml (3). The gallbladder is

divided into three regions, the fundus, the body, and the neck, which joins the cystic duct and ultimately combines with the common hepatic duct to form the common bile duct. The common bile duct joins with the main pancreatic duct to drain into the duodenum This terminal segment is known as the ampulla of Vater. It is surrounded by smooth muscle, the sphincter of Oddi Delivery of bile into the duodenum is not only controlled by contraction of the gallbladder but also by the motility of the sphincter of Oddi (4)

The gallbladder wall is composed of five layers, the innermost mucous layer, the lamina propria, the muscular layer which consists of irregularly oriented smooth muscle fibre bundles, the perimuscular connective tissue layer, and an outermost serous layer.

The blood supply of the human gallbladder usually arises from a single cystic artery, a branch of the right hepatic artery There are numerous anatomic variations which are of greater surgical importance than physiologic significance. The organ also receives blood directly from the hepatic surface of the liver. There is no major cystic vein Venous return runs either directly to the liver or toward venous radicals from the common bile duct Lymphatic channels accompany the veins The gallbladder receives both sympathetic and parasympathetic nervous supply. These originate both in the celiac plexus and the vagus nerves.

The gross anatomy of the guinea pig biliary system is very similar to that of the human The guinea pig gallbladder is also pear-shaped, and its relationships to the liver, ductal system and duodenum are the same as in man (5) The volume of the organ is much smaller, varying from 0 8 to 1.5 ml There is even

more variability in the arrangement of the blood supply than in man. As in man the muscle layer is composed of muscle fiber bundles running at various angles (5). The innervation is also similar to that in man (6).

The human gallbladder has two functions Its mucosa absorbs water and electrolytes resulting in concentration of bile. The gallbladder itself serves for storage and delivery of this bile (7). While the guinea pig gallbladder has the same storage and delivery functions as in the human, unlike most other species, it does not concentrate bile

Under normal circumstances, food in the duodenum causes the gallbladder to contract (8) The intraluminal pressure rises forcing bile into the common bile duct and, after relaxation of the sphincter of Oddi, bile will flow into the duodenum (9,7) Both these effects, contraction of the gallbladder and relaxation of the sphincter of Oddi are stimulated by postprandial cholecystokinin release (7,9,10,11,12,13,14). Delivery of bile into the duodenum is dependent on the dynamics of gallbladder contraction, the contractile state of the sphincter of Oddi, and the secretory rate of bile from the liver.

Gallbladder motility is under neural and hormonal control. A cephalic phase of neural control has only recently been described and was not previously thought to exist (9) despite analogous responses in the esophagus and stomach. It has been shown that the human gallbladder can start to empty prior to food reaching the duodenum and can even contract and empty in response to a sham meal (15). These contractions are completely abolished by atropine and

not associated with a rise in plasma cholecystokinin (16), evidence for a cholinergic mechanism, perhaps under parasympathetic control.

The gallbladder undergoes spontaneous contractions that are associated with cyclic motor activity in the esophagus and stomach (17) These spontaneous contractions are associated with specific phases of the migrating motor complex (18). Contractions induced by exogenous cholecystokinin have not been shown to be related to the migrating motor complex

The neural control of gallbladder motility can be divided into parasympathetic and sympathetic influences Parasympathetic influences on gallbladder motor activity mainly regulate resting tone while the major role of the sympathetic system seems to be to inhibit gallbladder contraction (7,9)

Parasympathetic stimulation, either pharmacologic (19) or through direct electrical stimulation of the nerves (20) increases the tone and motility of the gallbladder. However, this has not been shown to cause any ejection of bile Total vagotomy leads to an enlargement of the gallbladder (21) yet gallbladder emptying in response to exogenous cholecystokinin or meals seems to be unaffected (22) or in fact increased, perhaps due to denervation supersensitivity (9) or the absence of an intrinsic inhibitory inervation of the gallbladder (23)

The sympathetic influence on the gallbladder is more complex Gallbladder smooth muscle contains both excitatory and inhibitory adrenergic receptors (24). Injection of norepinephrine or direct sympathetic nerve stimulation can only relax the gallbladder if its tone is increased over baseline (7)

Within the gallbladder there is another network of nerve fibers containing peptides such as vasoactive intestinal polypeptide, substance P, somatostatin, metenkephalin and bombesin (25) These are intermingled with nerves containing the classical neurotransmitters of the sympathetic and parasympathetic systems These newly described nerve pathways may be involved in mediating the effects of exogenously administered peptides on gallbladder motility which had been previously ascribed to a hormonal action (26,27)

The most important factors regulating gallbladder motility are hormonal (7). Cholecystokinin is the primary physiologic stimulant of postprandial gallbladder contraction in animals, humans, and even in pathological states such as in patients with gallstones (12,13,14). Cholecystokinin is released from the endocrine cells of the small intestine in response to digestion products, notably phenylalanine, tryptophan, fatty acids, and hydrogen ions (9) The carboxy terminal octapeptide of cholecystokinin was found to be even more potent than the whole molecule (28,29). The cholecystokinin induced gallbladder contraction is independent of the autonomic nervous system, it is not affected by atropine, nor histamine, nor blocked by alpha or beta adrenergic blockade, nor is it affected by depolarizing agents or tetradotoxin (7). Presumably, there are unique cholecystokinin receptors on gallbladder smooth muscle (30,31). There may also be cholecystokinin receptors located on myenteric nerves (27,31) and cholinergic neurons acting to potentiate the effect of cholecystokinin (10,11,30).

Several other hormones also act on the gallbladder. Gastrin has structural similarities to cholecystokinin It can also cause gallbladder contraction though much less potent than cholecystokinin. The dose of gastrin required to

stimulate gallbladder contraction greatly exceed the dose necessary to stimulate gastric acid secretion (9).

Secretin and its related peptides glucagon and vasoactive intestinal polypeptide can also modulate gallbladder contractility (9). Secretin cannot alone cause gallbladder contraction but may potentiate the effect of cholecystokinin (32) This effect requires greater doses of secretin than are required to stimulate pancreatic secretion, the primary known physiologic function of secretin.

Glucagon can increase the size of the gallbladder in humans but only in pharmacologic doses (33). Vasoactive intestinal peptide is not released postprandially yet it is postulated that it may modulate postprandial gallbladder tone by acting as a neurotransmitter following vagal stimulation (9) Vasoactive intestinal peptide relaxes the cholecystokinin stimulated gallbladder (34)

Many more hormones play a role in gallbladder motility. It is currently believed that while cholecystokinin may regulate postprandial gallbladder contraction, interdigestive gallbladder activity may be regulated by other factors such as motilin (35). With *in vitro* bioassays it can be shown that the majority of the gallbladder contracting bioactivity of fasting human serum remains after removal of cholecystokinin (36). *In vitro* rabbit studies have suggested that serotonin may play a role in resting gallbladder tone and in interdigestive contractions (36). To make sense of the multitude of hormones found active in various *in vitro* animal studies it must be remembered that not all these substances may be equally active *in vivo* or in normal human physiology

Gallbladder disease is common in western society, where 16 to 20 million people in the United States are estimated to have gallstones (37). Yearly 300 000 to 500 000 cholecystectomies are performed with attendant morbidity and loss of time from work. Mortality is fortunately very low (37). The current understanding of gallstone formation is that it is a disease of the gallbladder (38).

Gallstone formation is a multiphasic process (39). The first step is the production of bile that can lead to stone formation. When the concentration of cholesterol relative to the amount of bile salt and lecithin exceeds a threshold the bile is supersaturated and gallstones can form (40). Progression of supersaturated bile to gallstones requires nucleation. This can occur spontaneously or may be due to the presence of bacteria, parasites or desquamated epithelial cells (41). Cholesterol stones often have pigment stone centers (39). Nucleation can be accelerated by inflammation (41). Nucleation and growth of the stone requires time. Some decrease in gallbladder motility is needed. In animal models supersaturated bile will not form gallstones if the gallbladder is regularly emptied by the administration of cholecystokinin (42). The gallbladder has a major role in gallstone formation at several levels (38).

Gallstones affect women at least twice as often as men (37,43). In radiologic studies, male gallbladders tend to empty more rapidly than those of females (44). The risk of gallstone disease increases with the number of pregnancies (43). Female sex hormones can affect gallbladder motility (1,45,46,47). The incidence of cholecystectomies increases with the use of supplemental estrogen (48). The prevalence of gallstone disease increases with age (43). Experimentally the *in vitro* gallbladder contractile response to cholecystokinin

decreases with age (49), perhaps due to changes in the cholecystokinin receptor with aging (50). A subgroup of patients with gallstones proven at laparotomy have abnormal gallbladder motility in response to cholecystokinin in preoperative radiologic evaluation (47). In another study, patients with gallstones were shown to have abnormal contractility in response to a meal while the response of their gallbladders to exogenous cholecystokinin was the same as controls (51). In prairie dogs and squirrels, this decrease in gallbladder motility occurred before the formation of gallstones (52) An understanding of gallbladder motility is important in elucidating the pathogenesis of gallstone disease.

Patients with gallstones most often present with pain (37), usually epigastric or right upper quadrant that often radiates to the subscapular or infrascapular region. Zollinger (53) placed balloon catheters into the gallbladders and common bile ducts of female patients with gallstones proved at laparotomy. These patients were awakened during the operation and the balloon was used to dilate either the gallbladder or the common bile duct. He found that gallbladder distension alone cause epigastric discomfort similar to the symptoms the patients had described preoperatively. Common bile duct distension was even more painful.

In a similar study, fluid was injected into the choledochostomy tubes of patients after cholecystectomies for gallstones (54). Flow of fluid and pressure were measured while inquiring about symptoms. Common bile duct distension caused deep epigastric or right upper quadrant pain Reflex spasm of the sphincter of Oddi occurred when the pressure in the common bile duct was suddenly increased. This was very painful for the patients. Thus, the

modulation of biliary motility is involved not only in the pathogenesis of gallstone disease, but also in its clinical manifestations.

Unlike skeletal muscle, the action potential of smooth muscle is calcium dependent (55). Excitation contraction coupling also requires calcium (56). Calcium is the mediator of the guinea pig gallbladder contraction caused by cholecystokinin (57,58). There are several pools of calcium available for muscle contraction. There is an extracellular pool and several intracellular pools. The intracellular pool can be further divided into cytosol, sarcoplasmic reticulum and mitochondrial pools (59).

The response of the muscle cell to membrane depolarization is due largely to the influx of extracellular calcium (60,61). However, the removal of extracellular calcium from the bathing medium during *in vitro* studies does not completely abolish contraction (57,62), demonstrating the contribution of intracellular pools of calcium Chlorpromazine, at low doses, decreases the influx of extracellular calcium and blocks the potassium depolarization induced contraction of the gallbladder but does not completely block the cholecystokinin induced contraction (63). Intracellular calcium depletion, or the inhibition of calcium ion storage in the sarcoplasmic reticulum significantly decreases agonist induced isolated gallbladder muscle strip contraction while the potassium depolarization induced contraction remains unaffected (64).

With depolarization of the muscle cell membrane, calcium enters the cell through several channels (58). There are voltage dependent and receptor linked calcium channels (59). These can further be divided into tonic and phasic channels. The tonic channels are involved in resting muscle tone while

the phasic channels respond to changes in voltage and to agonists to cause muscle contraction (59).

A high cholesterol diet has been shown to decrease the contractile response of *in vitro* gallbladder muscle strips from prairie dogs and ground squirrels (52,65) Pregnancy also causes decreased contractility of *in vitro* gallbladder strips (62). These studies suggest that either membrane channels or contractile protein synthesis or function are under sensitive control.

Nifedipine is a calcium channel blocker used widely in the treatment of cardiovascular disease. Nifedipine is one of a group of drugs synthesized to resemble papavarine, an alkaloid with vasodilator properties. Calcium channel blocking drugs act by binding to the calcium channels themselves (66) They decrease smooth muscle contraction as well as cellular secretion (67) There is evidence that the inhibition of the phasic channels is noncompetitive while that of the tonic channels is competitive (68) There is significant intra and interindividual variability in the clearance and bioavailability of calcium channel blockers (66,69). Therefore, no effective therapeutic plasma concentration range has been established. The hemodynamic side effects of nifedipine are influenced by the pharmaceutical preparation and the rate of drug administration (69). Nifedipine can be administered in a fixed dose rather than a dose per kilogram body weight. The dose administered should be titrated to the clinical effect. There are no equivalent dosages between routes of administration.

Calcium channel blocking drugs have been shown to relax smooth muscle (67,70). These drugs are used clinically in the treatment of coronary artery

disease and hypertension (70). Studies of the calcium channel blocker verapamil in the opossum esophagus *in vitro* and *in vivo* have shown that calcium channel blockers can decrease lower esophageal sphincter tone (71). This has been confirmed in normal humans and in patients with the nutcracker esophagus (72,73). Calcium channel blockers have been shown to inhibit bladder detrusor contractions in rabbits (68). Both verapamil and nifedipine inhibit potassium induced contractions of *in vitro* guinea pig ileum strips (74,75). Verapamil inhibits the stimulatory effects of substance P on intestinal secretion in dogs (76). Calcium antagonists do not block gastric emptying in humans (77,78) yet will inhibit antral motility (78). Nifedipine is useful in the treatment of the irritable bowel syndrome by decreasing bowel motility (67).

Cholecystokinin may cause gallbladder contraction and sphincter of Oddi relaxation by an action at three different sites, directly on the smooth muscle cells themselves, on the postganglionic cholinergic neurons, and on postganglionic noncholinergic, nonandrenergic inhibitory neurons (10,11,27,30,31). The contribution of each site may well depend on the experimental model chosen. There is no doubt that gallbladder contractility is mediated by calcium (55,57,58). We hypothesize that calcium channel blockers will inhibit cholecystokinin induced gallbladder contractility. Calcium channel blockers are able to modify gastrointestinal contractility both *in vitro* and *in vivo* and even in human subjects. Calcium channel blockers should inhibit cholecystokinin induced gallbladder contraction in human subjects.

Methods:

Animal Studies

Experimental Model:

Female Hartley guinea pigs weighing approximately 500 g (range, 470 to 600), were briefly anesthetized with ether and their spinal cords were sectioned at T1-T2 by electrocoagulation. The spinal cord was then packed with compresses to further block conduction. This rendered the animals pain free and immobile. They were then allowed to recover from anesthesia so that the experiments could be performed on awake, unanesthetized animals This is important because anesthesia has been shown to affect biliary motility (63,80) Polyethylene catheters (PE 50, Clay Adams, Parsippany, NJ) were inserted into the right jugular vein and carotid artery for venous access and blood pressure monitoring respectively.

Gallbladder pressure was measured using a technique developed in our laboratory and previously described (1,2). Microballoon catheters were constructed of polyethylene tubing with a single side hole and capped with a 1 cm balloon made of condom rubber (Ramses Sensitol, Julius Schmidt, Canada) filled with saline. The abdomen was opened with a 1 to 1 5 cm right paramedian incision. The gallbladder was always just below this incision and could easily be delivered through the wound. Gallbladder contents were aspirated with a fine needle. A microballoon catheter inflated with 0.25 ml of saline was inserted into the fundus of the gallbladder for pressure recordings The catheter was secured with a silk tie carefully placed to ensure a tight seal around the catheter, while including a minimal amount of gallbladder tissue within the suture. The cystic duct was not ligated. The nerve and blood supply

remained intact. The abdomen was then closed. The animals were kept in a thermoregulated water bath at 38 °C for the duration of the experiment. The experimental design was approved by the McGill University and the Montreal General Hospital Animal Care Committees.

Gallbladder intraluminal pressure and systemic arterial pressure were recorded using pressure transducers (Model P23AC, Southam Instruments, Hato Rey, Puerto Rico) connected to a polygraph (Model 7B, Grass Instruments, Quincy MA). The gallbladder pressure was electronically integrated (Polygraph Summating Integrator Model 7P10, Grass Instruments, Quincy MA), providing a measure of gallbladder contraction dependent on both the amplitude and the duration of gallbladder contraction.

Experimental Design:

During the baseline period prior to the administration of nifedipine, cholecystokinin octapeptide (Sigma Chemical Co., St. Louis, MO) was administered as an intravenous bolus of 20 ng/kg in 100 μL volume every 20 minutes. The resultant gallbladder contraction was recorded.

A dose of 20 ng/kg was given because this dose had been previously shown to cause an intermediate size gallbladder contraction in our model (1). Intervals of twenty minutes between cholecystokinin injections were chosen to allow for the known half-life of cholecystokinin in plasma (81) and to allow for sufficient time for gallbladder tone to return to baseline. The cholecystokinin was kept on ice for the duration of the studies, and all tubing and syringes were flushed after

each dose with 0.5 % bovine serum albumin (Sigma Chemical Co., St. Louis, MO) to prevent adherence of the cholecystokinin to the tubing.

The administration of cholecystokinin was not timed to the migrating motor complex despite the probable importance of the migrating motor complex in spontaneous gallbladder contractions and the possibility that postprandial gallbladder contractions may be linked to the migrating motor complex (18). We have shown in a previous study using this model that there was a high correlation between contractile response and cholecystokinin dose, $r > 0.95$ (1). This implies that the measured response to cholecystokinin can almost entirely be ascribed to the dose of cholecystokinin.

The mean baseline gallbladder contractile response was calculated from the response to four doses of cholecystokinin prior to the administration of nifedipine. Nifedipine (Bayer Pharmaceuticals, Montreal, PQ) was then slowly infused intravenously over 10 minutes in a dose of 100, 200 or 300 µg Each dose was tested on a separate group of five guinea pigs.

We used a fixed dose of nifedipine for several reasons There was little variation between animal weights. There is no correlation between biological effect, serum levels and dose of nifedipine (66,69,82). Furthermore, there is not only interindividual variation but intraindividual variation for the same dose and route of administration (69).

Care was taken to prevent exposure of nifedipine to light. As in the baseline part of the experiment, cholecystokinin-octapeptide was administered every

20 minutes for 130 minutes after starting the nifedipine infusion, and the gallbladder contractile response was measured.

Parameters of gallbladder contractility which were measured (see figures 1 and 2) included: 1) amplitude of contraction, which is the peak pressure rise in response to the cholecystokinin injection; 2) the integrated contractile response or area under the pressure-time tracing, thus reflecting both the amplitude and duration of the contraction, 3) the rate of rise of gallbladder pressure (dP/dT), a measure of contractility; 4) the rate of spontaneous gallbladder contractions in the unstimulated state; 5) resting gallbladder pressure, reflecting the tone of gallbladder wall.

Data Analysis:

Data for each parameter of gallbladder contraction were expressed as mean ± SEM. The contractions induced by cholecystokinin immediately after nifedipine were compared to the mean of the baseline contractions before nifedipine by the paired "t" test, with each animal serving as its own control. Analysis of variance was used to demonstrate an effect on gallbladder pressure over time for each dose of nifedipine. A p value of less than 0.05 was considered significant.

Human Studies

Nine healthy human volunteers with normal gallbladder ejection fractions (greater than 50 %) were studied. Each subject gave informed consent for this study which was approved by The Montreal General Hospital Clinical Trials Committee. The subjects were all in good health, on no medications, and had no gastrointestinal symptoms Eight were male, one female, and the mean age was 25 years.

After an overnight fast, each volunteer underwent a baseline radionuclide study of gallbladder emptying in response to the synthetic cholecystokinin octapeptide sincalide (Kinevac, Squibb, Canada Inc , Montreal, PQ) Five millicuries of technetium-99m-labeled diisopropyliminodiacetic acid prepared from a commercial kit (Disofenin, DuPont, Lachine, PQ) was injected as an intravenous bolus. After allowing at least one hour for washout of background liver activity and good visualization of the gallbladder, the anterior image was recorded on computer, forming the baseline. Immediately afterwards, 20 ng/kg of cholecystokinin in 5 ml of saline were infused into an antecubital vein continuously over 15 minutes. Gallbladder activity was monitored continuously for 30 minutes with a gamma-ray scintillation camera and the information was stored in a computer at one frame per minute On playback, windows were placed manually over the gallbladder image and carefully adjusted in each frame to exclude extraneous radioactivity in the gut and the common bile duct as gallbladder emptying progressed The gallbladder ejection fractions relative to baseline were thereby calculated for each time frame The maximum ejection fraction over the 30 minute recording session was used in the final analysis. A repeat cholescintiscan study of the gallbladder emptying was performed on

another day 30 minutes after a 10 mg oral dose of nifedipine (Miles Laboratories, Etobicoke, ON).

Data Analysis:

The maximum gallbladder ejection fraction after the oral dose of nifedipine was compared to the maximal ejection fraction of the baseline study by the "t" test for paired data. A p value of less than 0.05 was considered significant. Gallbladder ejection fractions for each group were expressed as mean ± SEM.

Results:

Animal Studies:

The resting gallbladder undergoes six to eight spontaneous contractions per minute (Figure 1). Administration of nifedipine caused an immediate dose independent reduction of this rate to less than one spontaneous contraction per minute. This was associated with a decrease in the resting gallbladder tone of up to 10 cm H_2O (Figure 1). Spontaneous gallbladder contractions had not returned by 130 minutes after completion of the nifedipine infusion

Cholecystokinin induced an immediate gallbladder contraction with a rapid rate of rise of gallbladder pressure and a characteristic waveform as shown in Figure 2. The amplitude, integral, and rate of pressure rise were stable both over time and between animals. The administration of nifedipine caused a significant time-limited reduction in all parameters of this response

As shown in Figure 3A, nifedipine 100 μg caused a 45 % ($P < 0.01$) reduction in the amplitude of gallbladder contraction Nifedipine 200 μg resulted in a 73 % ($P < .01$) reduction and 300 μg a 67 % ($P < 0.01$) reduction These effects decreased progressively over time By ANOVA there was a significant difference between the lower dose of nifedipine and the two higher doses, but there was no significant difference between the two higher doses for the effect on amplitude.

The integrated contractile response is shown in Figure 3B There were significant ($P < 0.01$) reductions in the integrated gallbladder contractile

responses at the 10 minute interval of 68, 62, and 77 %, for the 100, 200, and 300 μg doses of nifedipine, respectively, compared to the mean basal response

Figure 3C shows that dP/dT was not significantly decreased in response to the lowest dose of nifedipine; however, the rate of rise of gallbladder pressure was significantly decreased in response to the two higher doses ($P < 0.01$). Nifedipine 200 μg caused a 43 % decrease in the dP/dT. While there was a significant difference between the 100 μg dose of nifedipine and the higher doses, there was no significant difference between the two higher doses.

The administration of nifedipine caused mild, transient hypotension. The hypotension was related to the rate of administration of the nifedipine and had resolved before the effect on gallbladder contraction was recorded. In a group of four animals that received a 200 μg dose of nifedipine there was an initial 25 % transient decrease in mean systemic arterial pressure from a mean of 60.8 ± 7.7 to 45.9 ± 9.1 mmHg. At the time of the first cholecystokinin stimulated contraction 10 minutes after nifedipine administration the mean arterial pressure had returned to 53.9 ± 7 There was no relation between this hypotension and the effect of nifedipine on gallbladder contractility. To confirm this, in two separate animals, hemorrhage resulting in a sustained 29 % decrease in mean arterial pressure did not change the mean amplitude of cholecystokinin-induced contraction from 20 to 22 cm H_2O.

Human Studies:

The ejection fraction for our healthy volunteers in the baseline study was 72 ± 5 %. After the 10 mg oral dose of nifedipine the mean ejection fraction decreased to 51 ± 5 %(Figure 4). This is a significant decrease of 29 % ($P < 0.001$). All volunteers demonstrated some decrease in their ejection fraction with nifedipine, and as seen in Figure 5, the decrease in ejection fraction is nearly parallel in most of the subjects

There was no hypotension measured in the subjects 30 minutes after ingestion of nifedipine. The mean systolic/diastolic arterial pressures prior to the administration of nifedipine were 118 ± 9.8/68 ± 5 mmHg and following nifedipine were 117 ± 9/64 ± 18 mmHg. Two of the nine volunteers complained of headaches after taking nifedipine; one of these subjects complained of headaches during both scans. It was not clear wether this might have been due to the fasting required for the study. No other side effects were noted.

Figure 1:

Spontaneous gallbladder contractions are seen as small peaks during the baseline period. This activity is abolished by the administration of nifedipine and there is a resultant decrease in the resting gallbladder tone.

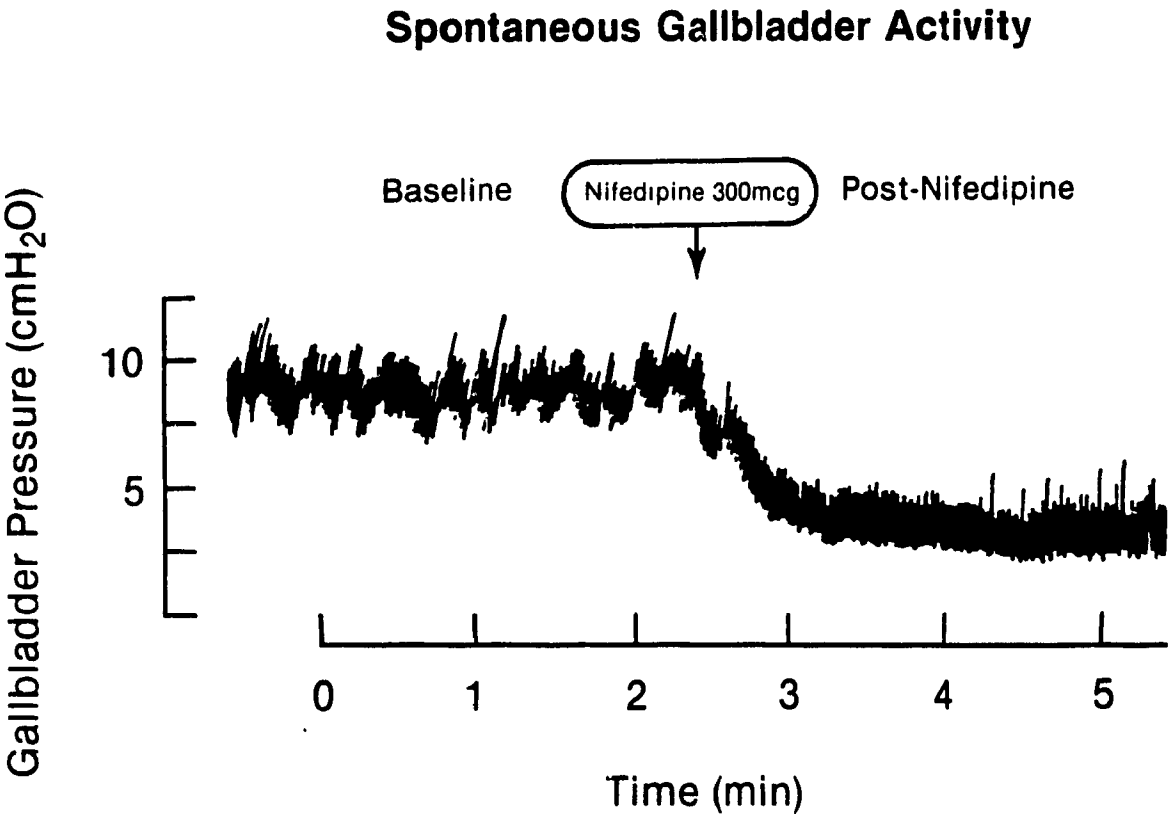


Figure 2:

The characteristic waveform of the gallbladder pressure response to a 20 ng/kg dose of cholecystokinin is shown. The amplitude of the pressure developed by a gallbladder contraction is labelled The time to develop maximal pressure is t The dP/dt is calculated from these values and represents the rate of rise of gallbladder pressure during contraction. The shaded area under the curve represents the electronically integrated area.

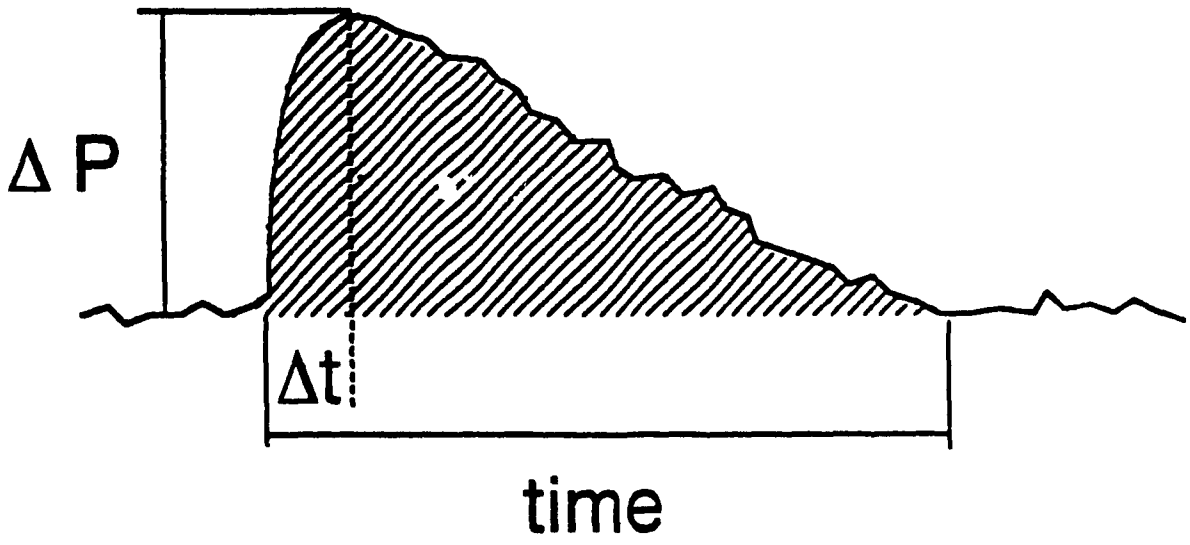


Figure 3:

A The amplitude of gallbladder pressure response to cholecystokinin is shown before and after nifedipine. Each column represents mean ± SEM of five guinea pigs. There was a significant ($P < 0.01$) decrease 10 minutes after nifedipine for all doses tested.
B The integrated contractile response to cholecystokinin is shown before and after nifedipine. There was a significant decrease 10 minutes after nifedipine for all doses tested.
C The rate of gallbladder pressure (dP/dt) in response to cholecystokinin is shown before and after nifedipine. There was a significant decrease in dP/dt 10 minutes after nifedipine in response to only the 200 and 300 µg doses of nifedipine.

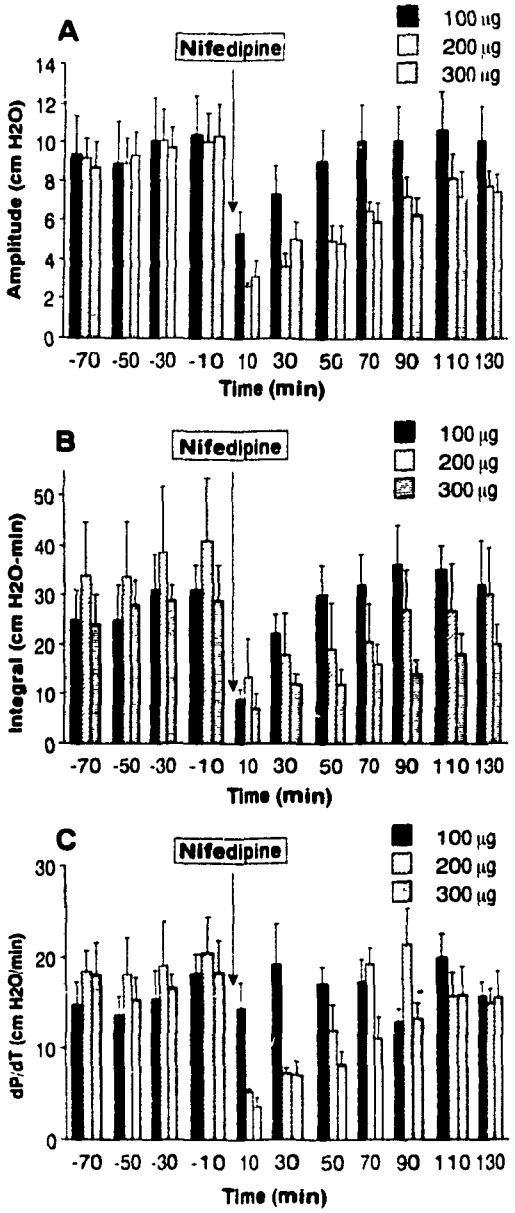


Figure 4:

The percent gallbladder ejection fraction (mean ± SEM) for nine healthy volunteers is shown for baseline and nifedipine studies. There was a significant decrease in gallbladder ejection fraction after nifedipine

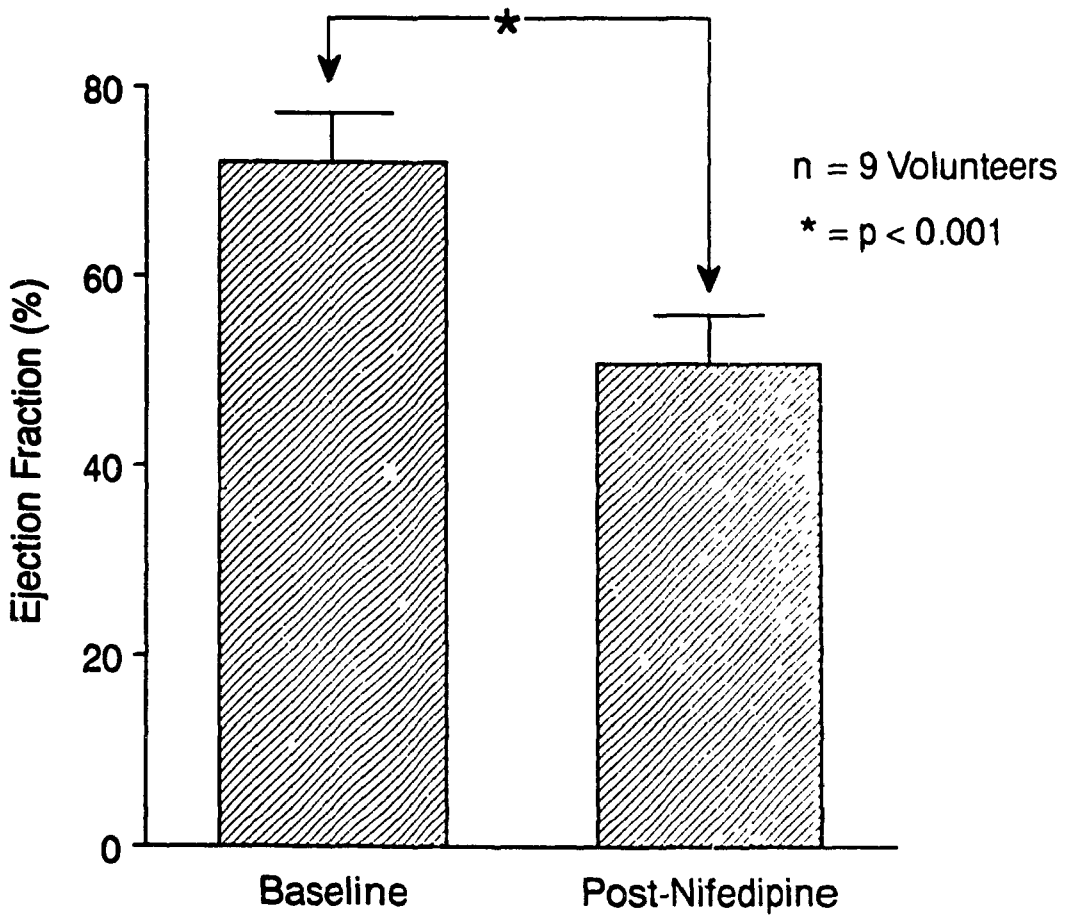
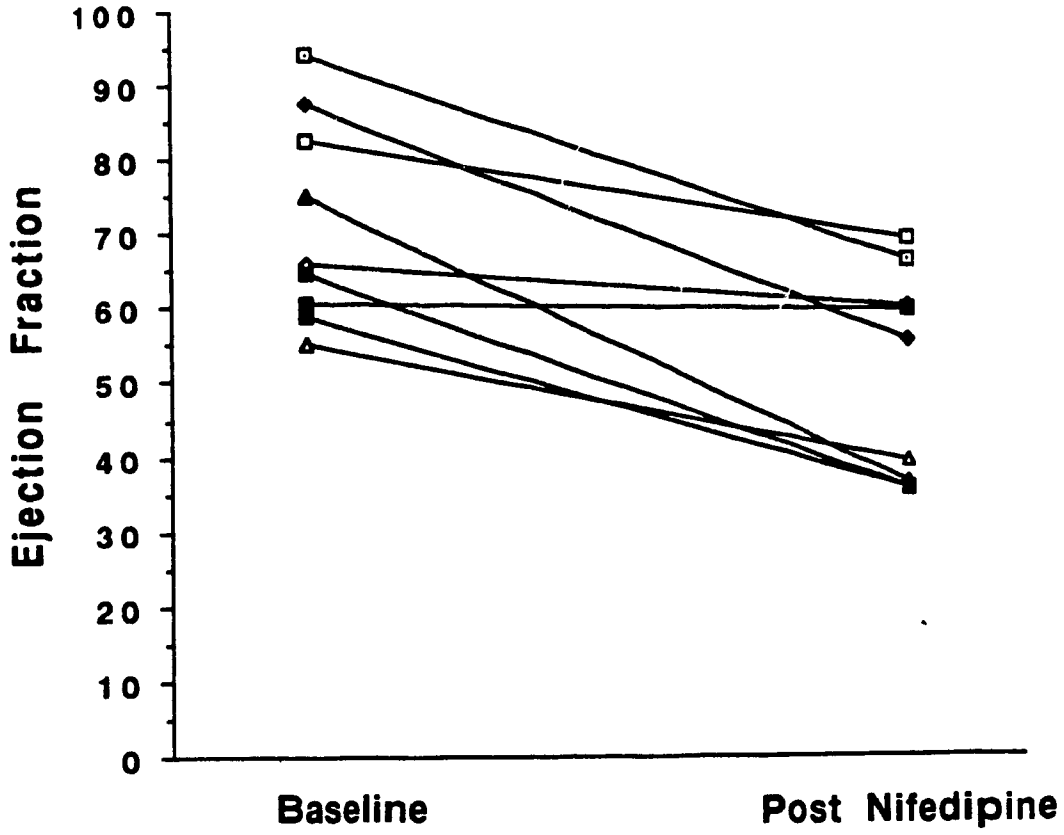


Figure 5:

The percent gallbladder ejection fraction for each of the nine volunteers is shown for baseline and nifedipine studies. All volunteers demonstrated a decrease in gallbladder emptying. The decrease was nearly parallel for most of the subjects.



Discussion:

In this study we used an invasive *in vivo* method to measure gallbladder contractility in the conscious guinea pig. We used noninvasive radionuclide cholescintigraphy to record gallbladder contractility in man.

There exist several *in vitro* and *in vivo* models for evaluating gallbladder contractility (4,83,84,85,86,87,88,89,90,91). *In vitro* methods have the great advantage of allowing the isolation of a single factor to assess its importance Unfortunately, the effects of that factor *in vitro* may be very different *in vivo* where interactions with the normal regulatory mechanisms of gallbladder contractility occur. *In vivo* methods are only valuable in so far as the normal physiology of the biliary system remains intact. *In vivo* methods can be divided into noninvasive and invasive techniques.

Noninvasive *in vivo* techniques to record gallbladder activity use radiologic methods, today primarily ultrasound to record gallbladder size in two dimensions (92), and radionuclide cholescintigraphy, utilizing density of radioactivity to evaluate three dimensional size (4). Changes in volume of the gallbladder are interpreted to represent changes in contractility. These techniques have been applied to animals however this remains difficult, thus fraught with error. These techniques cannot be applied to small rodents and remain expensive and complicated.

Several invasive *in vivo* models have been described (83,84,87) including some that allow for chronic studies (87,89). All these techniques are based on either perfusion of the gallbladder (85,86,87,89,90) with recording of flow and

pressure, direct gallbladder tension measurement (83,88), or intravesical balloons (1,84).

Perfusion techniques have been used in chronic studies and this is a great advantage (87,89,90). Unfortunately, this technique requires ligation or stenting of the sphincter of Oddi to measure gallbladder contractility alone. The blood and nerve supply of the gallbladder run close to the cystic duct (6) with the possibility of injury Without manipulation of the sphincter, the technique measures combined gallbladder and sphincter activity.

Direct measurement of gallbladder tension utilizes simple equipment (83,88). Unless the gallbladder is securely anchored inaccuracies are introduced. Anchoring the gallbladder can put traction on ductular, vascular and neural structures affecting the behavior of the physiologic system. Suturing to the sternum has been used (83), however this greatly magnifies respiratory artifacts. With direct measurement only changes in organ length can be recorded. Changes in diameter or isovolumetric contraction cannot be recorded Asymmetric contractions introduce errors.

Intravesicular balloons are simple and inexpensive to construct or purchase. The recording equipment is simple, easy to obtain and comparatively inexpensive. The technique can be applied to many organ systems (93). These techniques have been used for a long time. Zollinger used an intravesicular balloon in his 1933 human gallbladder studies (53).

The guinea pig gallbladder intravesicular microballoon model used in this study has many advantages. As discussed, the anatomy of the guinea pig is

remarkably similar to that of man. The animals are inexpensive, straightforward to obtain, are docile when handled, and easy to house

The microballoons were made by us of inexpensive materials, polyethylene tubing and small pieces of condom rubber (1,2,93). These are long lasting, in fact most of this study was performed using the same balloon Use of the balloon does not require ligation of the cystic or common bile duct and thus there is no risk of damaging any intrinsic biliary control pathways Recorded pressures are not dependent on sphincter of Oddi activity as no bile flow is necessary. In fact, the balloon fills the gallbladder and there is no bile accumulation. The balloon induces a constant gallbladder shape and volume. This ensures consistency between animals. Gallbladder pressures recorded in this and previous studies are similar (1,2).

No anesthesia was used during the experiment. Cervical section ensured that the animals would feel no pain. Cervical section could produce effects on the gallbladder similar to vagotomy. It has been shown that the gallbladder effects of vagotomy in man and in animals are not immediate, requiring several months to result in loss of basal tone and gallbladder dilatation (9,94)

In our *in vivo* model, using an awake animal, we have demonstrated that the calcium channel blocker nifedipine can decrease cholecystokinin-induced gallbladder contraction in a time-limited manner. While there is a relationship between the magnitude of the effect and the dose of nifedipine used, it would appear that the maximal effect is achieved with an intravenous dose of 200 μg. This decrease was manifested by significant reductions in gallbladder contraction amplitude and integrated contraction, which is a measure of both

amplitude and duration of contraction. The dP/dT, an estimation of contractility, was decreased by the two larger doses of nifedipine, but was not affected by the lower dose despite the significantly decreased gallbladder contraction amplitude induced by the lower dose. These effects could not be attributed to the mild transient hypotension occasionally observed after nifedipine.

Gallbladder emptying has been described in coordination with the intestinal migrating motor complex in other models. We have not recorded any substantial changes in intracholecystic pressure in our model, or shifts in baseline pressure, other than those changes occurring directly in response to the bolus injection of cholecystokinin. Furthermore, changes in the gallbladder contractile response to cholecystokinin after nifedipine were sufficiently uniform between animals to imply that the migrating motor complex would not have significantly affected our measurements of gallbladder contractility with this model.

Several noninvasive methods of measuring gallbladder motility in man have been described. The oldest and most common method is the oral cholecystogram (9,34,44,93). The gallbladder is opacified and ejection monitored with serial anteroposterior and lateral radiographs taken at timed intervals. Gallbladder volume is calculated based on measurements of the radio-opaque areas in the serial radiographs. Several mathematical models exist for the calculation of volume from two dimensional images.

Ultrasonography is also based on planimetric measurements of serial anteroposterior and lateral images of the gallbladder (93). It has the advantage of reduced radiation exposure which introduces the possibility of continuous

gallbladder recording. Both radiologic and ultrasonographic methods are limited by the need to transfer two dimensional measurements into volume using theoretical mathematical models based on constant gallbladder shape during contraction.

Radionuclide cholescintigraphy is simple, relatively inexpensive, and widely available. Continuous recording of gallbladder volume is possible with only low doses of radiation. No theoretical mathematical interpolation are required, gallbladder volume is directly related to the density of radioactivity recorded (44).

The baseline gallbladder ejection fraction in response to cholecystokinin measured by cholescintigraphy in our human study correlates well with measurements of ejection fractions published in other series (95,96) Oral nifedipine (10 mg), when given to normal volunteers, caused a significant reduction in the gallbladder ejection fraction. The trial of nifedipine in volunteers demonstrated that this drug can also be used orally to decrease gallbladder emptying in humans.

Cholecystokinin induces gallbladder contraction primarily by causing an increased entry of extracellular calcium into smooth muscle (57). Removal of calcium from the incubation medium causes a greater that 90 % reduction in cholecystokinin induced gallbladder muscle strip contraction (62). Calcium chelators have the same effect (58). Our results *in vivo* in both guinea pigs and man seem to support these findings. However, our study did not address the mechanism of cholecystokinin action. This question could be further addressed with our animal model by investigating the action of other calcium channel

blockers and also calcium channel activators such as BAY K8644 (91). There may be evidence, based on voltage clamp membrane studies, that nifedipine can block contraction independently from its action on calcium channels (97).

Cholecystokinin is the primary physiologic stimulant of postprandial gallbladder contraction (13,) A correlation between plasma cholecystokinin concentration and gallbladder contractility has been shown in healthy people and in patients with gallstones (14) Cholecystokinin receptor blockade causes dose-dependent inhibition of postprandial gallbladder contraction (98). Since nifedipine is effective in decreasing cholecystokinin induced gallbladder contraction, it should be able to inhibit normal postprandial gallbladder contractions.

Ceruletide is a decapeptide isolated from frog skin that has numerous structural similarities with cholecystokinin(9). In certain animal models, ceruletide has been shown to be as much as ten times more potent than cholecystokinin in inducing gallbladder contraction (9).

In one study it was demonstrated in man that nifedipine did not block ceruletide induced gallbladder contraction measured by ultrasound (79). The dose of ceruletide used was low, 2 ng/Kg/min, a dose more likely to cause relaxation of the sphincter of Oddi. The ceruletide induced sphincter of Oddi relaxation can cause passive gallbladder emptying. This would mask any possible decrease in gallbladder contractility *in vivo*. It has been shown *in vitro* that calcium channel blockers will decrease ceruletide induced isolated gallbladder strip contraction (99). This aspect of ceruletide induced gallbladder contraction should form the basis of further study.

Gallbladder contractility in patients with gallstones is related to plasma concentrations of cholecystokinin (14). It seems likely that the pain of biliary colic is due to contraction of the obstructed gallbladder in response to release of endogenous cholecystokinin Based on our experimental data we feel that there may be clinical relevance for the use of nifedipine in the treatment of biliary colic. It may be possible to alleviate the pain that these patients experience with the use of oral or sublingual nifedipine. The nonoperative management of gallstones has been proposed with the advent of oral cholelitholytic agents and lithotripsy. Nifedipine may potentially be of use in these patients to control pain of biliary colic while awaiting the resolution of their stones.

Some success in reducing the pain of biliary colic has been shown using drugs that can decrease gallbladder contractility. Indomethacin reduces raised intraluminal gallbladder pressure in acute cholecystitis (100,101). This was shown to decrease biliary pain in a double blind clinical trial (102) Low dose ceruletide, which relaxes the sphincter of Oddi without causing gallbladder contraction, has also been shown to be effective in decreasing biliary pain in a double blind placebo controlled trial (103).

Nifedipine is widely used for the treatment of cardiovascular disease If chronic use of this drug can be shown to persistently inhibit gallbladder contraction, the resultant stasis may result in gallstone formation. This may be analogous to the observed increased risk of' gallstones in patients on prolonged parenteral nutrition (104) who have been shown to have decreased gallbladder

contractility on radionuclide cholescintigraphy (82). These areas are the subjects of ongoing studies.

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