Arris & Gale Lecture

Regulation and responses of gallbladder muscle activity in health and disease

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A complex relationship links biliary symptoms with the mechanisms of gallbladder emptying and the presence of gallstones. This relationship has been investigated by clinical studies of symptoms associated with gallstones, by investigation of gallbladder emptying and cholecystokinin (CCK) release in patients with gallstones, and after cholecystectomy, or truncal vagotomy, and in the irritable bowel syndrome (IBS). Laboratory studies examined receptor density on bovine gallbladder, and contractility of human gallbladder in response to a variety of stimuli was studied *in vitro*. A set of six symptoms associated with the presence of gallstones was identified; IBS appeared to be present in two-fifths of patients before cholecystectomy but only one-third of these patients had persistent IBS symptoms one year after operation. Gallbladder emptying studies confirmed the poor contraction of stone-bearing gallbladders; abnormal patterns of emptying were also found in patients with IBS. Gallbladder emptying and filling appear to be largely neurally regulated. CCK receptor density was very low in gallbladder tissue, suggesting that receptors on nerve cells might mediate the action of CCK. Relaxation of gallbladder muscle was mediated by adrenergic and nitrergic nerves. The inflammatory mediator bradykinin, however, had a strong direct action on muscle cells to cause gallbladder contraction. The work reported here gives an overview of the symptoms and mechanisms of disease associated with the presence of stones in the gallbladder.

Key words: Biliary symptoms – Gallbladder emptying – Gallstones – CCK release – Cholecystectomy

This article describes several strands of research which all throw light on our understanding of the relationships between symptoms of biliary and other abdominal disorders, the formation of gallstones and their effect on the gallbladder, the normal mechanisms of regulation of gallbladder muscle activity, and how these mechanisms may be impaired in the presence of gallstones. The work falls into three main areas: (i) the study of symptoms

related to gallstones; (ii) studies of gallbladder emptying; and (iii) *in vitro* investigation of regulation of gallbladder muscle contraction (Fig. 1).

At the end of the 1980s, it was supposed that in the absence of acute cholecystitis, biliary symptoms could arise either from obstruction to flow during gallbladder contraction, producing biliary colic, or gallstones producing irritation and inflammation of the gallbladder wall leading

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Clinical studies **Gallbladder emptying studies** **In vitro studies**

Figure 1 Summary of the work presented here, and the main points which arise.

to the symptoms of biliary dyspepsia. Gallbladder emptying was believed to consist of a sustained gallbladder contraction in response to a meal. During fasting, the gallbladder filled

(and bile became more concentrated by absorption of water) and after eating the gallbladder emptied. These concepts have turned out to be gross simplifications.

Symptoms in patients with gallstones

The prevalence of gallstones in the UK population is high: 8% of individuals over the age of 40 years will be affected.¹ It has been noted that the 6-month prevalence of dyspeptic symptoms is 41% and that one-quarter of affected individuals will have consulted a doctor.² However, only about 10% of gallstones are symptomatic,¹ so it follows that there will be considerable overlap of non-specific symptoms and the presence of innocent gallstones.

The symptoms of 'gallstone dyspepsia' were described in 1975.³ However, others^{4,5} have found that dyspepsia was no more common in individuals with gallstones than in the general population and these 'gallstone dyspepsia' symptoms are now largely discounted. By contrast, symptoms associated with gallstones include constant abdominal pain of at least 30 min duration, and radiating to the upper back,⁶ and severe radiating abdominal pain, a history of previous attacks and intolerance to fatty foods.⁷ Right upper quadrant pain alone was not significantly associated with gallstones.⁶ In studies of randomly selected individuals, there have been no obvious differences in the incidence and nature of abdominal pain and other symptoms between those who do and do not have stones.^{8,9}

Another important observation is that patients who undergo cholecystectomy for the relief of biliary symptoms are often disappointed. Ros and Zamboni¹⁰ found that half the patients they investigated 2 years after cholecystectomy experienced mild but distressing symptoms. Many of these patients appeared to have functional gastrointestinal disorders unrelated to the presence of the gallstones.

We have investigated symptoms in patients referred for investigation of possible gallstones by ultrasonography, to identify any association between symptoms and gallstones in this selected group. Separately, symptoms before and after cholecystectomy were studied to determine whether any specific pre-operative symptoms were more likely to persist after cholecystectomy.

Symptoms in patients suspected of biliary disease

Patients ($n = 174$) who underwent ultrasound examination for investigation of symptoms were studied. All of these had pain for at least 30 min duration during the previous month. Gallstones were diagnosed in 45 patients and 10 were diagnosed with some other condition and were excluded from the study. Before ultrasonography, the patients completed a detailed symptom questionnaire.¹¹ Multivariate analysis was conducted to identify symptoms associated with the presence or absence of gallstones. In this group of patients undergoing

Table 1 Symptoms associated with the presence or absence of gallstones (Harper¹¹)

	Gallstones likely to be present	Gallstones likely to be absent
Severity of pain	Severe	Mild/moderate
Pain associated with vomiting	No	Yes
Age (years)	> 60	< 60
Acid reflux	Yes	No
Sharp pain	No	Yes
Jaundice	Yes	No

investigation for suspected gallstones, four symptoms and severe pain were significantly associated with the presence of stones and the description of pain as 'sharp' was significantly associated with the absence of stones (Table 1). In a subsequent validation study of 126 patients, the presence of four or more of these factors correctly predicted 19 of 28 patients with stones, but the negative predictive value (*i.e.* ability to identify patients without gallstones correctly) was not sufficient to dispense with the ultrasound investigation: stones were found in 9 of 79 patients predicted to have no stones (pre-test likelihood of no stones 75%, post-test likelihood 89%).

Symptoms before and after cholecystectomy for gallstones

In an initial sample of 50 patients studied just before cholecystectomy, symptoms of flatulent dyspepsia and distension were more common than in patients presenting for ultrasonography. The striking observation was that the prevalence of irritable bowel syndrome (IBS) symptoms as defined by the Manning criteria¹² was 32% before cholecystectomy. However, when these patients were investigated again 1 year after operation, two-thirds of patients who had IBS symptoms before cholecystectomy were symptom-free 1 year later (Table 2). In a further study to determine whether the response to cholecystectomy was long lasting, nearly one-quarter of 61 patients interviewed 5 or 6 years after cholecystectomy reported significant abdominal pain. Only 6 patients (10%) fulfilled the criteria for IBS.¹⁴

These findings did not support our initial supposition that pre-operative symptoms of IBS might persist and so

Table 2 Prevalence of irritable bowel syndrome symptoms before and after cholecystectomy (from Jones¹³). Two of 50 patients were lost to follow-up.

Absent (–) pre-operatively	28
Present (+) pre-operatively	22
+ to +	6
+ to –	16
– to –	24
– to +	2

account for the high proportion of patients with symptoms after cholecystectomy. It is possible that symptoms confidently attributed to IBS can be relieved by cholecystectomy in a substantial proportion of patients, and that this effect is relatively long-lasting as the proportion of patients reporting IBS 5 or 6 years after operation remained low (6 of 61 patients). The hypothesis that poor gallbladder emptying may lead to reduced inhibition of cholecystokinin (CCK) release, and that higher levels of CCK might, in turn, lead to painful stimulation of colonic contraction mimicking IBS symptoms, is examined below.

Gallbladder emptying and cholecystokinin

Patients with gallstones show abnormal patterns of gallbladder emptying.^{15,16} Situations in which gallbladder emptying is impaired predispose to gallstone formation.¹⁷ We were, therefore, interested in determining the most appropriate methods for assessment of gallbladder emptying, and to investigate whether it was possible to promote gallbladder emptying by therapeutic manipulation.

Radioisotope imaging

This technique relies on the principle of intravenous administration of a radioisotope-labelled bile salt, which is trapped in the liver and excreted into the bile. The radioisotope accumulates in the gallbladder and then, after administration of a stimulus, the decay in the signal from the gallbladder region of interest can be measured over time. This technique is well described and is known to be reliable.^{18,19} Using a new radioisotope (^{99m}Tc -BrIDA) and a dual-headed gamma-camera capable of acquiring simultaneous anterior and posterior images, we showed that a stable plateau was reached 70–90 min after administration of the radioisotope in unstimulated subjects.²⁰ After stimulation with a fatty meal, there was a smooth and progressive ejection of radioactivity from the gallbladder region of interest with a median ejection fraction at 80 min of 51% (range, 34–76%) and levelling off at approximately 60% after 90 min.

However, Howard and colleagues²¹ have shown that gallbladder emptying is not a smooth progression, but rather consists of alternating phases of filling and emptying. Real-time ultrasonography has shown that there is an early phase of rapid emptying followed by a refilling phase at approximately 45 min after the meal. There is then a late period of emptying, usually to less than 30% of the fasting volume in normal subjects. Furthermore, they showed that there are large minute-to-minute changes in gallbladder volume, suggesting that gallbladder emptying proceeds by a sequence of contractions and relaxations with intermittent

Figure 2 A representative example of a gallbladder emptying profile in a normal volunteer in whom measurements of gallbladder volume were made at 1 min intervals. Reproduced with permission from Howard *et al.*²¹

refilling (Fig. 2). It is suggested that this pattern of emptying helps to dilute concentrated gallbladder bile and flush it out of the gallbladder, thereby removing small cholesterol crystals and reducing the risk of stone formation.

Because isotope methods can only demonstrate the emptying of bile from the gallbladder and take no account of gallbladder refilling, we elected to use ultrasound measurement of gallbladder volume to investigate gallbladder emptying. It has been shown that there is a correlation between fasting gallbladder volume and residual volume;²² data are reported as the percentage reduction in gallbladder volume after the stimulus (ejection fraction: $[\text{residual volume}/\text{fasting volume}] \times 100\%$).

Manipulation of gallbladder emptying

Several factors influence gallbladder emptying; truncal vagotomy has been widely studied. Some authors have reported reduced gallbladder emptying after truncal vagotomy,^{23–26} whereas others have found normal values.^{27,28} We believe these discrepancies may arise from differences in the timing of investigation after operation and in the different methods used for estimating gallbladder function. There was modest increase in fasting volume in our patients who had undergone truncal vagotomy, compared with healthy volunteers (Fig. 3).²⁹ This was associated with a reduced ejection fraction (63% [range 51–68%] after vagotomy; normal 76% [range, 58–91%]).

We attempted to modify gallbladder emptying in normal subjects and in vagotomised patients by administering cisapride 10 mg, 4 times daily. The prokinetic agent cisapride

Table 3 Release of CCK after a standard meal in normal subjects and after truncal vagotomy with pyloroplasty (TVP), or pyloroplasty alone

	Control	Pyloroplasty	TVP
Fasting CCK	0.57 (0.31–1.15)	0.86 (0.56–1.04)	1.48 (0.92–2.29)*
Peak CCK	5.62 (2.49–10.84)	7.28 (4.52–12.68)	12.92 (9.12–25.69)*
Integrated CCK	300 (161–643)	333 (190–380)	588 (308–1122)*

Values are pmol, median and range. Data from Patankar *et al.*³³

* $P < 0.05$ against control and pyloroplasty (Mann-Whitney test).

has been shown to increase contraction of gallbladder muscle strips in response to electric field stimulation *in vitro*.³⁰ One report had suggested that this drug could increase gallbladder emptying,³¹ although this had not been confirmed.³² In our study,²⁹ no significant change was seen in fasting volume or ejection fraction during treatment with cisapride 10 mg 4 times a day, either in healthy subjects or vagotomised patients. Indeed, residual volume was increased which suggests that any prokinetic effect of cisapride on the gallbladder is masked by other effects, perhaps increased tone at the sphincter of Oddi.

A possible explanation of the differences in gallbladder volumes after vagotomy could be explained by changes in CCK release. However, after vagotomy, CCK release was greater than in normal subjects (Table 3), suggesting that the impaired gallbladder emptying was due to causes other than changed CCK release. Indeed, impaired gallbladder emptying might contribute to increased CCK release by reduction of a negative feedback effect. CCK release was normal after pyloroplasty alone, without vagotomy (performed for the management of bleeding duodenal ulcer; Table 3), ruling out the possibility that more rapid gastric emptying was responsible for the increased CCK release.

Gallbladder emptying, CCK release and gallstones

It has been known for some time that patients with gallstones show two distinct patterns of gallbladder emptying. The minority demonstrate normal gallbladder emptying, whereas most patients show poor or absent contraction.^{15,34} Masclee *et al.*³⁵ and van Erpecum *et al.*³⁶ have shown reduced gall bladder emptying in some gallstone patients, together with increased fasting and residual gallbladder volumes. However, not all studies have concurred (for review, see Patankar *et al.*¹⁷) justifying further studies.

In any consideration of gall bladder emptying, CCK release plays an important part. Changes in gall bladder emptying can only be understood with simultaneous measurement of CCK release, as noted above with post-vagotomy subjects. Gallbladder emptying is related to CCK release, apparently by a negative feedback mechanism.

Figure 3 Gallbladder emptying after a standard meal in patients after truncal vagotomy and normal subjects. Reproduced from Patankar *et al.*²⁹

Reduced gall bladder emptying in the presence of normal or raised CCK secretion suggests a defect of contraction in the gallbladder itself, whereas reduced contraction with diminished CCK release would suggest a reduced stimulus to the gallbladder.

Gallbladder emptying and CCK release were studied in 6 normal subjects and 27 with gallstones. Of the latter, 10 patients had no discernible gallbladder emptying, and the remainder could be divided into good contractors ($n = 9$) and poor contractors ($n = 8$) as defined by Thompson *et al.*,³⁴ i.e. outside the 95% confidence interval of the normal subjects. The good contractors showed a similar pattern of gall bladder emptying to the normal subjects (Fig. 4).

Table 4 CCK release after a standard stimulus, in normal subjects, gallstone patients and after cholecystectomy. Data from McDonnell *et al.*³⁸

	Peak CCK	Integrated CCK
Controls	2.6 (1.1)	8.35*
Gallstone patients	3.8 (1.2)	14.73
After cholecystectomy	4.9 (1.1)	20.44*

* $P < 0.05$ difference between these groups (Mann-Whitney test).

Figure 4 Gallbladder emptying in normal subjects and in patients with gallstones, divided into good contractor and poor contractor groups. Data from Bailey.³⁷

Fasting plasma concentrations of CCK were similar in normal subjects, patients with gallstones, and after cholecystectomy.³⁸ However, the meal-stimulated plasma CCK was significantly elevated after cholecystectomy compared with normal subjects (Table 4), and there was a trend to higher CCK levels in patients with a non-functioning gallbladder compared to those with stones and normal gallbladder function.

CCK and irritable bowel syndrome

It is known that CCK can stimulate colonic contraction, and can cause pain which mimics the pain of IBS. Further, as noted above, there are similarities between the symptoms of some patients with gallstones and IBS. Gallbladder motility and CCK release were studied in gallstone patients and in patients with IBS.³⁹ These studies show that, in 28 patients with a functioning gallbladder, the majority ($n = 20$) had poor contraction. These patients had a higher peak CCK value after stimulation than normal individuals. Interestingly, the gallbladder emptying pattern in IBS was variable, with 2 of 11 patients showing very poor emptying and the remainder emptying more vigorously than the normal subjects. CCK values in IBS were also greater than in normal individuals, both fasting and after stimulation, suggesting that in this condition increased gallbladder emptying might be due to increased release of CCK. This leads to the proposal that increased CCK release may be a primary feature of IBS. It

is difficult, however, to explain the abolition of IBS symptoms in patients undergoing cholecystectomy for symptomatic gallstones.

From receptacle to receptors

The work described above is concerned with the physiological function of the gallbladder and how it might be affected in various disease states. CCK and neurotransmitters exert their effect on the gallbladder muscle cells by interaction with specific receptors. Gallbladder muscle will respond to a variety of stimuli, particularly the neurotransmitter acetylcholine and adrenergic transmitters, in addition to CCK. It has been suggested that CCK may not act directly on the gallbladder muscle, but by the intermediary of enteric nerves, perhaps by receptors on ganglion cells within the gallbladder neural plexus.^{40,41}

It is also clear that regulation of gallbladder muscular activity could involve a relaxation, as for prolonged periods the gallbladder passively dilates to accommodate bile secreted from the liver. There is clearly a reciprocal interplay between the gallbladder and the sphincter of Oddi, as stimuli which cause gallbladder muscle contraction lead to relaxation of the sphincter, and *vice versa*, and neural connections between the two, and between the gallbladder and the duodenum are known to exist.⁴² Furthermore, inflammatory mediators such as bradykinin are known to have a direct action on smooth

Figure 5 Receptor binding of CCK8 to membrane preparations of bovine pancreas, brain and gallbladder. Data from Miarkowski.⁴⁸

muscle in other sites, such as the intestine⁴³ and in the gallbladder in the guinea pig.⁴⁴ The remainder of this paper describes a number of *in vitro* studies we have performed to investigate these aspects of gallbladder muscle contraction.

CCK receptors

Decreased contraction of the gallbladder in patients with gallstones could be explained by reduced sensitivity to stimulation as a result of changes in the expression of CCK receptors on the muscularis. Some authors have demonstrated apparent binding to smooth muscle by radiolabelled CCK analogues.^{45,46} Von Schrenck *et al.*⁴⁷ have shown that the characteristics of CCK receptors on gallbladder and pancreas were similar. Therefore, we attempted to quantify the numbers of receptors expressed on purified plasma membranes of tissues known to be responsive to CCK, *i.e.* pancreas, brain and gallbladder. Receptor density was calculated by Scatchard analysis, using CCK 8 and radiolabelled Bolton-Hunter CCK 8 to determine binding.

Strong specific binding was observed with pancreatic plasma membranes, indicating a receptor density greater than 600 fmol/mg protein. Binding was much less on brain plasma membranes, with a calculated receptor density 6.5 fmol/mg protein. Binding to gallbladder plasma membranes was considerably lower than this, and very high membrane protein concentrations were required to obtain specific binding in excess of the non-specific binding (Fig. 5). Receptor concentrations for gallbladder muscularis could not be calculated.

These observations confirm the very low CCK receptor density suggested by others.^{15,49} One interpretation of these findings would be that CCK acts directly on pancreatic acinar cells, but the very much lower receptor density in brain and gallbladder muscularis would be consistent with action mediated by neuronal stimulation.

Receptive relaxation

In the early 1990s, the concept of relaxation mediated by the neurotransmitter nitric oxide changed our understanding of many physiological processes. Nitric oxide was proposed as a mediator for the non-adrenergic, non-cholinergic (NANC) inhibitory or relaxatory nerve fibres that have been demonstrated in the gastrointestinal tract, and in guinea pig gallbladder.⁵⁰

We obtained samples of functioning human gallbladder from operative specimens and suspended these in a tissue bath between electrodes. In this set-up, application of an electrical field at appropriate frequency and duration can depolarise neurons without causing direct muscle stimulation. Under cholinergic blockade, this neurally mediated stimulation could produce a relaxation of human gallbladder muscle.⁵¹ In the presence of atropine and guanethidine, to produce cholinergic and adrenergic blockade, and after stimulation of contraction with CCK 8, electric field stimulation produced a repeatable relaxation of the gallbladder muscle strips. We showed that this could be inhibited by the addition of L-nitro-arginine, suggesting that the relaxation of gallbladder muscle was mediated by nitric oxide.⁵¹

Subsequently, it has been suggested that nitric oxide donors might be clinically useful for the treatment of painful biliary colic.⁵²

Inflammatory mediators

In the experiments described above, there was significant correlation between the amount of inflammatory infiltrate observed on histology and the ability of gallbladder muscle to contract to a standard stimulus.⁵³ It seemed appropriate, therefore, to investigate the effects on gallbladder muscle contractility of bradykinin, a well-known inflammatory mediator which has varied effects on intestinal smooth muscle contractility.

Initial experiments showed a dose-dependent stimulatory effect of bradykinin on human gallbladder muscle.⁵⁴ A series of experiments was performed to investigate the nature of bradykinin-induced gallbladder muscle contraction.⁵⁵

In normal gallbladders, the bradykinin-stimulated contraction is mediated by two receptors (BK1 and BK2). In stone-bearing gallbladders, the BK1 receptor appears to be down-regulated as BK1 receptor antagonists no longer

influence the action of bradykinin. Simultaneous blockade of BK1 and BK2 receptors produces no additional inhibition and, at high concentrations of bradykinin, there was still a response. Although this persisting response could reflect competitive inhibition, with stimulation occurring in the presence of excess bradykinin, there was not a corresponding increase in inhibition when increasing concentrations of BK1 and BK2 receptor antagonists were administered in conjunction with a constant submaximal concentration of bradykinin. This leaves open the possibility that a further receptor to bradykinin is involved in the response.

Further studies have shown that these actions of bradykinin are mediated by direct intracellular signalling, involving calcium and the cyclooxygenase pathway, and further work is in progress to determine interactions between bradykinin and CCK.

Conclusions

These studies reveal some useful insights into the symptoms of a number of gastrointestinal disorders. However, we have been unable to clarify the relationship between various symptoms and the presence of gallstones, and so have not yet been able to suggest ways to reduce the frequency of residual symptoms after cholecystectomy. Changes in CCK release may have a bearing on the increased severity and frequency of gastro-oesophageal reflux symptoms after cholecystectomy³⁸ and abnormalities of gallbladder emptying and CCK release in IBS suggest an exciting avenue for further investigation and possible therapeutic studies in this common disorder.

Gallbladder function is generally represented as the reception of bile, as a passive process, and its subsequent ejection as a result of stimulation of muscle contraction. This work has shown that understanding of this activity requires an appreciation of the method of study (measurement of bile ejection or gallbladder volume), and consideration of the interaction between gallbladder emptying and CCK release. Investigation of receptive relaxation as a neurally mediated active process led to the interesting observation that the contractile ability of the gallbladder is related not only to the strength of stimulus with physiological agents such as CCK, but also to the amount of inflammatory infiltrate in the gallbladder wall. Changes occur in bradykinin receptors in stone-bearing as opposed to normal gallbladders, raising the possibility of an interaction between normal stimuli and inflammatory mediators such as bradykinin in gallbladder contraction. These observations may explain the finding that cyclooxygenase inhibitors, such as indomethacin, can affect gallbladder function and the recurrence of gallstones after dissolution therapy.⁵⁶⁻⁵⁸

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