Nerves and Hormones Interact to Control Gallbladder Function

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Ganglia are the target of several regulatory inputs to the gallbladder. Hormonal cholecystokinin and sympathetic nerves can up- or downregulate neurotransmission in the gallbladder, respectively, by altering the rate of acetylcholine release from vagal preganglionic terminals. Peptides released from sensory axons act directly on gallbladder neurons to increase their excitability.

In the 1920s, Ivy and Oldberg demonstrated that gallbladder emptying involves the release of a hormone from the intestinal mucosa, and they named the yet to be isolated compound cholecystokinin (CCK) for its stimulatory effect on the gallbladder. Despite the fact that we have accepted this fundamental property of gallbladder function for decades, the actual mechanisms that lead to hormonal and neural regulation of gallbladder function are not fully understood. These gaps in our knowledge regarding gallbladder function are especially surprising given the fact that the direct costs associated with biliary malfunction are greater than those associated with any other gastrointestinal ailment, including ulcer disease or colorectal cancer (National Institutes of Health publication no. 94–1447).

A major reason for our lack of knowledge about the specifics of gallbladder motility is that the nerves that lie within the wall of the organ have remained a veritable black box. Therefore, data regarding the neural components of a given phenomenon have been obtained indirectly, often with pharmacological agents whose precise site(s) of action could not be clearly resolved. Although these approaches generated considerable evidence for the importance of the nervous system in normal gallbladder function, it was impossible to produce a detailed description of
where a given compound was acting or how the gallbladder's neural components function. This has changed in recent years as a handful of laboratories, including our own, have conducted electrophysiological and immunohistochemical studies that focus on the ganglia that lie within the wall of the gallbladder. Results of these studies indicate that gallbladder ganglia are the target of modulatory hormonal, sympathetic, and visceral afferent signals that influence gallbladder muscle and epithelial activity. The purpose of this paper is to discuss the evidence that has accumulated in support of the concept that CCK acts largely through a neural mechanism to cause gallbladder emptying and to provide an overview of how other inputs to, and outputs from, gallbladder ganglia could influence gallbladder function.

Properties of gallbladder ganglia

The muscular and epithelial tissues of the gallbladder are innervated by neurons that are located in a ganglionated plexus that lies at the interface between the muscularis and serosal layers of the organ (see Ref. 14). It is likely that these neurons are derived from the same precursors, the vagal crest cells, that give rise to the enteric nervous system. The ganglionated plexus of the gallbladder is composed of an array of small, irregularly shaped ganglia that are arranged in no discernible order (Fig. 1A). The ganglionated plexus is contiguous with perivascular nerve bundles that follow blood vessels into the organ. The muscularis and mucosal layers also contain nerve fibers that represent a mixture of projections from intrinsic neurons, sympathetic postganglionic fibers, and sensory fibers. In some species, such as humans and the Australian possum, a sparse distribution of small ganglia is located in the mucosal plexus.

Intracellular recording techniques have been used to study the physiological properties of neurons in gallbladder ganglia of the guinea pig (5) and opossum (1). Gallbladder neurons of these species have phasic properties; i.e., they fire only one to a few action potentials at the onset of a prolonged depolarizing current pulse, regardless of the amplitude or duration of the current pulse, and spontaneous activity is rarely observed. These characteristics indicate that gallbladder neurons only fire action potentials when they receive excitatory signals such as those from the...
vagal efferent fibers and therefore only release their neurotransmitters onto target tissues in response to incoming commands.

Two types of excitatory synaptic inputs have been detected in gallbladder ganglia, fast excitatory synaptic potentials (EPSP) and slow EPSPs. The fast EPSPs, which are mediated primarily by vagal input, can be subthreshold events or they can result in action potentials, whereas the slow EPSPs are typically not associated with neuronal firing. Therefore, with regard to the generation of output from gallbladder ganglia, the vagal preganglionic axons represent the principal driving force. As described below, the efficacy of this ganglionic relay can be up- or downregulated by physiological signals that act presynaptically on vagal terminals or postsynaptically on gallbladder neurons. No inhibitory synaptic events have been encountered in gallbladder ganglia.

**Gallbladder ganglia are a target of hormonal CCK**

Although the concept that CCK acts as a hormone to cause gallbladder emptying is solidly established, the means by which this occurs has been the subject of considerable debate. Substantial support exists for the conventional view that CCK acts directly on smooth muscle cells to cause gallbladder contractions, but growing evidence suggests that a neural mechanism is more important physiologically. An example of the confusion that has existed in this area is found in the literature describing investigations of gallbladder function, in experimental animals and humans, following vagotomy (see Ref. 11 for a review). In support of the view that CCK acts through a neural mechanism, some reports indicate that following vagotomy there is 1) a decrease in gallbladder emptying after meals or in response to CCK, 2) an increase in resting gallbladder volume, and 3) an increased incidence of stone formation. In support of a direct action of CCK on gallbladder muscle, intravenous infusion of CCK causes gallbladder contraction following vagotomy, and, in some cases, no change in postprandial gallbladder emptying was observed. Unfortunately, it is difficult to draw a definitive conclusion from these studies because of the variability that exists in their approaches and data collection. For example, the extent of the vagotomy is crucial because the gallbladder is supplied by the hepatic branch, and studies have been conducted at various time points following vagotomy.

Other approaches that have been used to explore this issue include in vivo experiments with cholinergic blocking agents and in vitro experiments on gallbladder muscle strips. Several studies have demonstrated that muscarinic blockers, which are likely to act at the junction between gallbladder neurons and smooth muscle, significantly attenuate food-induced gallbladder contractions as well as CCK-induced gallbladder contractions. This indicates that CCK is somehow causing an increase in the release of acetylcholine from gallbladder neurons and, in turn, an increase in gallbladder tone. Consistent with this view are reports that CCK causes the release of acetylcholine from gallbladder muscle strips. On the other hand, when applied to gallbladder muscle strips, CCK causes a concentration-dependent increase in tension that is not affected by neural blockade with muscarinic antagonists or the sodium channel blocker, tetrodotoxin. Furthermore, receptor autoradiography and assays for CCK receptor messenger RNA have confirmed the expression of CCK-A receptors by gallbladder smooth muscle.

Taken together, the evidence summarized above indicates that CCK receptors are present on gallbladder muscle but that CCK may act at least in part through a neural mechanism to stimulate gallbladder emptying following a meal. Fundamental questions that could help resolve the issue of the site(s) of the physiological action of hormonal CCK in initiating gallbladder emptying include the following: 1) do the nerves of the gallbladder respond to CCK, and if so, how? and 2) what are the relative sensitivities of neural and direct muscle responses to CCK, and how do these correlate with serum concentrations of CCK following a meal?

The actions of CCK in gallbladder ganglia have been studied by applying CCK to whole mount preparations while recording from individual neurons with intracellular microelectrodes. Results of these studies, which have been conducted in the guinea pig (6, 9) and the opossum (1), indicate that CCK can act presynaptically in gallbladder ganglia to increase synaptic input to gallbladder neurons, but it does not have a direct effect on gallbladder neurons. In other words, CCK acts on cholinergic nerve terminals to increase the amount of acetylcholine released each time an action potential reaches that terminal. The outcome of this presynaptic facilitory effect is that the amplitude of the fast EPSP is increased, usually converting subthreshold events to suprathreshold EPSPs, resulting in the release of neurotransmitter onto the muscle. In a series of experiments that involved eliciting synaptic events by stimulating nerve bundles that pass along the cystic duct before and after vagotomy, we demonstrated that the principal source of cholinergic input to gallbladder neurons is...
from vagal efferent fibers and that vagal inputs are sensitive to CCK (Fig. 2; Ref. 9).

One of the difficulties in accounting for a direct action of CCK on gallbladder muscle is that the concentrations of CCK that are required to contract gallbladder muscle strips are orders of magnitude higher than the serum concentrations of CCK following a meal. Whereas postprandial CCK levels in the serum are in the 10 pM range, the threshold for detection of increased gallbladder tension is in the 1–5 nM range, with maximal responses seen at 50–100 nM (eg., Ref. 4). Therefore, it was important to evaluate the concentration-effect relationship of the presynaptic response of CCK in gallbladder ganglia. This was not a simple task because addition of CCK to the bath, even at concentrations in the picomolar range, resulted in the generation of action potentials (Fig. 3) and therefore prevented quantification of the effect. To circumvent this problem, we used single-electrode voltage-clamp techniques to measure synaptic currents that resulted from acetylcholine release, while restraining voltage-activated currents. The synaptic current was increased by 20% in the presence of 10 pM CCK, indicating that gallbladder ganglia are a feasible site of action of CCK in physiological conditions (Fig. 3).

Over the past several years, studies involving in vivo preparations in guinea pig, opossum, and dog have provided additional support for the theory that the principal physiological effect of CCK in the gallbladder involves a neural mechanism (3, 10, 12). These studies demonstrated that gallbladder contractions elicited by feeding or by intravascular injections of physiological postfeeding concentrations of CCK were disrupted by atropine or cold block of the vagus nerves. Furthermore, nicotinic receptor antagonists, which act at the synapse between the vagal terminals and gallbladder neurons, also caused a marked reduction in the gallbladder responses. These data are consistent with the in vitro studies of gallbladder ganglia because they place a major

**FIGURE 2.** Synaptic responses elicited by electrical stimulation of vagal inputs to gallbladder neurons are enhanced by cholecystokinin (CCK) and are inhibited by norepinephrine (NE). A: subthreshold excitatory postsynaptic potentials (EPSPs) are converted to suprathreshold events in the presence of CCK. B: EPSPs evoked by cystic nerve stimulation are reversibly suppressed in the presence of NE. Each condition is represented by 5 consecutive events (A) or by an average of 5 consecutive events (B). [From Mawe et al. (9).]

**FIGURE 3.** Concentration of cholecystokinin (CCK) that is present in the serum after a meal is high enough to act in gallbladder ganglia but is too low to directly activate gallbladder muscle. Graphs represent the CCK-induced increase in synaptic current in guinea pig gallbladder and CCK-induced increase in tension of a guinea pig gallbladder muscle strip [Derived from data of Mawe (6), Harrington et al. (4), and Takahashi et al. (12).]
site of CCK's action proximal to the gallbladder neurons or muscle.

Given that CCK is probably acting through a neural mechanism to activate postprandial gallbladder emptying, there are two likely sites of action for this hormone. Both of these sites are in the peripheral nervous system, since CCK does not cross the blood-brain barrier. One potential site of action of CCK, as described above, is the vagus nerve terminals within gallbladder ganglia, where CCK has a potent facilitatory effect on transmitter release. The other likely site of action is vagal afferent fibers. Numerous studies have demonstrated that subdiaphragmatic vagal afferents are sensitive to CCK, and postprandial physiological responses such as increased gastric motility and pancreatic secretion have been attributed to CCK-mediated increases in vagal afferent activity. Our current working model is that, following a meal, CCK stimulates vagal afferent fibers, which could act in the vagal motor complex to increase the rate of firing of vagal preganglionic neurons. In addition, CCK acts in gallbladder ganglia to increase the amount of acetylcholine released each time the vagal motoneurons fire an action potential. The actions of CCK on gallbladder emptying would be compromised at both of these sites by muscarinic or nicotinic blockers or by vagotomy.

If CCK is acting physiologically on vagal afferent and efferent nerves, how does one explain the fact that meal-induced and CCK-induced gallbladder emptying still occurs following vagotomy? One likely mechanism for meal- and CCK-induced gallbladder emptying is through a decrease in the resistance to bile flow at the sphincter of Oddi. CCK has been shown to decrease sphincter of Oddi tone, and, unlike the gallbladder where neurons are unresponsive to CCK, sphincter of Oddi neurons are activated by this peptide. Gallbladder muscle cells exhibit spontaneous action potentials and myogenic tone; therefore, a decrease in resistance to bile flow at the level of the sphincter of Oddi would result in gallbladder contraction and emptying. Also, vagal denervation could result in sensitization of the muscle, resulting in a shift of the concentration-effect curve for CCK to a lower concentration range. Finally, gallbladder emptying following vagotomy could be mediated through an enterobiliary neural reflex. Studies in the guinea pig and the Australian possum have demonstrated that the gallbladder receives projections from neurons located in the myenteric plexus of the duodenum. It is plausible that luminal stimuli or mucosal CCK release could result in an activation of these neurons, resulting in the activation of a signal to the gallbladder.

**Other modulatory events that occur in gallbladder ganglia**

In addition to containing vagal preganglionic fibers that terminate on gallbladder neurons, the ganglionated plexus of the gallbladder contains rich networks of sympathetic postganglionic fibers that arise in the celiac ganglia as well as sensory fibers that arise in spinal and nodose ganglia. Because the adrenergic and sensory fibers exhibit numerous varicosities within the ganglia, which are likely to be sites of transmitter release, the possibility that these nerves could have targets in gallbladder ganglia has recently been explored.

In 1990, Yamasato and Nakayama (15) reported that subthreshold stimulation of the celiac nerve in the dog, which had no effect on gallbladder motility, markedly decreased the responsiveness of the gallbladder to vagal stimulation. These data indicate that sympathetic nerves may terminate, presynaptically, in the ganglionated plexus. Furthermore, these authors postulated that vagal input to the gallbladder could be modulated by activation of sympathetic postganglionic nerves. Subsequent studies in the guinea pig provide direct evidence for the existence of this type of circuitry (7, 9). Exogenously applied norepinephrine decreases the amplitude of fast EPSPs (Fig. 3) and converts suprathreshold EPSPs to subthreshold events in gallbladder ganglia by acting on \( \alpha \)-adrenoceptors. Furthermore, release of endogenous catecholamine stores, by tyramine application or by electrical stimulation of the vascular plexus, also causes a yohimbine-sensitive decrease in fast synaptic activity. Therefore, norepinephrine and CCK, which have opposite effects on the contractility of the gallbladder, both act presynaptically, and they have opposite effects on the release of acetylcholine from vagus nerve terminals.

The ganglionated plexus of the gallbladder is rich in varicose nerve fibers that are immunoreactive for both substance P (SP) and calcitonin gene-related peptide (CGRP) (Fig. 1B). These are probably extrinsic sensory fibers, since gallbladder neurons do not express CGRP and since coexpression of these peptides is a common characteristic of small-diameter axons that originate in sensory ganglia. We recently investigated the actions of tachykinins and CGRP in guinea pig gallbladder ganglia and found that both have a direct excitatory effect on gallbladder neurons (2, 8). Both depolarize gallbladder neurons and increase their excitability. Furthermore, antagonism of the neurokinin receptor that mediates the SP response, the neurokinin-3 receptor, attenuates capsaicin-induced depolarizations and slow EPSPs. This indicates that SP is released in gall-
bladder ganglia and is likely to mediate long-lasting excitatory synaptic events. The slow EPSPs are not accompanied by action potentials in these neurons, but the neurons are primed to respond more readily to other inputs such as those from vagal axons. These results indicate that the SP-CGRP-immunoreactive sensory fibers could act as the afferent limb of a local axon reflex circuit within the wall of the gallbladder. It is possible that, in response to inflammation or elevated intraluminal pressure, tachykinins and CGRP may be released within ganglia by sensory fibers and act directly on intrinsic neurons to facilitate ganglionic transmission.

### Outputs from gallbladder ganglia

To establish how gallbladder neurons can influence the muscle and mucosa of the organ, we have conducted a series of studies to determine which neuroactive compounds are expressed by gallbladder neurons. These investigations began with immunohistochemical studies in the guinea pig that demonstrated the existence of two major neuronal subpopulations in guinea pig gallbladder ganglia, based on their chemical coding (Fig. 1C; Ref. 13). One group, representing the majority of the neurons, expresses SP, neuropeptide Y, and somatostatin; SP and neuropeptide Y have been reported to contract gallbladder muscle. A distinct subpopulation of neurons expresses vasoactive intestinal peptide (VIP) and nitric oxide synthase (NOS); VIP and nitric oxide have been reported to relax precontracted muscle strips. Because of the chemical coding patterns that were observed and the actions of these compounds on gallbladder muscle, we proposed that the majority of neurons were excitatory neurons, and were probably cholinergic as well, and that the VIP-NOS neurons were not cholinergic and were inhibitory neurons. This theory has been largely invalidated by our recent findings in the guinea pig as well as the dog and human gallbladder.

Data from experiments involving the use of newly available antisera directed against choline O-acetyltransferase indicate that, as far as we can detect, all neurons in guinea pig, canine, and human gallbladders express choline O-acetyltransferase immunoreactivities and therefore are likely to synthesize acetylcholine (14). In the guinea pig, even the VIP-NOS neurons that were thought to be inhibitory are probably cholinergic (Fig. 1D). In the human and dog, the coexpression patterns are even more confusing, since, in addition to expressing the biosynthetic machinery to produce acetylcholine, the majority of neurons express immunoreactivities for VIP as well as SP. These patterns of coexpression bring about a dilemma when we attempt to contemplate an accurate model for the innervation of the gallbladder because many neurons in the system could themselves provide contradictory signals to a given tissue.

Several possible scenarios could explain the potentially opposing outputs from gallbladder neurons. One possibility is that excitatory and inhibitory neuroactive compounds are released from a given neuron under different circumstances in response to distinct types or sources of inputs. A second possibility is that compounds with opposing actions may be released onto the same target sequentially, with one acting as a physiological antagonist of the other. A third pos-
sibility is that both sets of compounds are released simultaneously, but they exert their effects on different targets. For example, if acetylcholine, SP, and VIP were released together, acetylcholine and SP could act on the muscle to cause a contraction while VIP acts on epithelial cells. Neuroactive compounds could also exert their effects on nearby nerve terminals to modulate further release of neuroactive compounds.

Concluding remarks

Gone are the days when autonomic ganglia were thought of as simple relay stations. The complex intrinsic reflex circuitry of the enteric ganglia represents an extreme example of this, but simpler systems such as the ganglionated plexus of the gallbladder are also sites of signal modulation. In the case of gallbladder ganglia, although the vagal input to postganglionic neurons appears to be the primary activator of ganglionic output, the efficacy of this circuit can be up- or downmodulated by various other signals that reach these ganglia (Fig. 4). For example, evidence suggests that hormonal CCK acts in gallbladder ganglia to activate gallbladder emptying following a meal. Release of neuroactive peptides from a sensory axon reflex circuit can also modulate gallbladder ganglionic output by enhancing the excitability of galbladder neurons. Given that gallbladder ganglia appear to be an important target of these physiological signals, it is likely that other agents that modulate gallbladder function, such as agents released during inflammation, could mediate their actions through a neural mechanism.

Output from the gallbladder ganglia involves the release of neuroactive compounds from intrinsic neurons. Acetylcholine, nitric oxide, and a number of neuroactive peptides can be synthesized by these neurons, and in this system it is common for single neurons to coexpress compounds that are reported to have opposing actions. Future investigations will be required to determine which neuroactive compounds are actually released by gallbladder neurons, which target cells in the gallbladder have receptors for the various neuroactive compounds, and which tissues are exposed to given compounds in the event of transmitter release.

Due to space and citation limitations, it was not possible to provide original citations for many of the studies that were alluded to here. Therefore, in line with the emphasis of the review, priority was given to citations related to studies of the neural control of gallbladder function. More comprehensive reference lists may be obtained in the bibliographies of the papers that are cited.

References