Smooth muscle cells, interstitial cells and neurons in the gallbladder (GB): Functional syncytium of electrical rhythmicity and GB motility (Review)

FAN DING[1,2*], QILI HU[3*], YIXING WANG[4], MIN JIANG[1,2], ZHENGYU CUI[4],
RUN GUO[5], LIPING LIU[5], FANG CHEN[5], HAI HU[1,2] and GANG ZHAO[1,2]

[1]Center of Gallbladder Disease, East Hospital of Tongji University, Shanghai 200120; [2]Institute of Gallstone Disease,
Tongji University School of Medicine, Shanghai 200331; [3]Department of Hepatobiliary Surgery,
The First People's Hospital of Hefei, Hefei, Anhui 230061; Departments of [4]Traditional Chinese Medicine
and [5]Ultrasonography, East Hospital of Tongji University, Shanghai 200120, P.R. China

Abstract. The motility of the gallbladder (GB) involves the storage, concentration and delivery of bile. GB motor functions are controlled by multiple complex factors, such as extrinsic and intrinsic innervation, humoral factors and neuropeptides. GB emptying results from coordinated contractions of the muscular layers of the GB wall. Depolarization of GB smooth muscle (GBSM) depends on the activation of the regular depolarization-repolarization potential, referred to as slow waves (SWs). These rhythmic SWs of GBSM contraction are mediated by several cell types, including smooth muscle cells (SMCs), GB neurons, telocytes (TC) and specialized pacemaker cells called interstitial cells of Cajal (ICC). The present article introduced a new GB motor unit, the SMC-TC-ICC-neuron (STIN) syncytium. In GB, STIN cells provide pacemaker activity, propagation pathways for SWs, transduction of inputs from motor and sensory neurons and mechanosensitivity. The present review provided an overview of STIN cells, mechanisms generating GBSM contractile behavior and GB motility, and discussed alterations of STIN cell function under different disease conditions.

Contents

1. Introduction
2. Morphology and distribution of STIN cells
3. GBSMs: Excitation-contraction coupling units
4. ICCs: Pacemaker of SWs
5. Telocytes: Purinergic inhibitory neurotransmission bridge
6. GB neurobiology
7. STIN syncytium and the pathophysiology of GB diseases
8. Conclusions

1. Introduction

The gallbladder (GB), an accessory organ of the gastrointestinal (GI) tract, stores and concentrates most hepatic bile

Correspondence to: Professor Hai Hu or Professor Gang Zhao, Center of Gallbladder Disease, East Hospital of Tongji University, 150 Jimo Road, Shanghai 200120, P.R. China
E-mail: huhailc@sina.com
E-mail: zhao_gang7@126.com

*Contributed equally

Abbreviations: GB, gallbladder; SMC, smooth muscle cell; TC, telocyte; ICC, interstitial cells of Cajal; ICLC, interstitial Cajal-like cells; GSMC, gallbladder smooth muscle cell; STIN, SMC-TC-ICC-neuron; GBSM, gallbladder smooth muscle; SW, slow wave; GI, gastrointestinal; PDGFRα+, platelet-derived growth factor receptor α-positive; SIP, SMC-ICC-PDGFRα+ cell; ENS, enteric nervous system; IHC, immunohistochemical; TEM, transmission electron microscope; α-SMA, α-smooth muscle actin; Ano1, anoctamin 1; NKCC1, Na+-K+-Cl-cotransporter; SR, sarcoplasmic reticulum; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; AP, action potential; VDCCs, voltage-dependent Ca^{2+} channels; ERG, ether-a-go-go-related gene; K$_{ATP}$, ATP-sensitive K+; BK$_{Ca}$, large-conductance Ca^{2+}-activated K; CGRP, calcitonin gene-related peptide; RyR, ryanodine-sensitive receptors; CCK, cholecystokinin; CCE, capacitative calcium entry; NSCCs, nonselective cation channels; TRPC, TRP protein family C; IP3R, inositol 1,4,5-trisphosphate receptors; GPCRs, G-protein-coupled receptors; CICR, calcium-induced calcium release; PKC, protein kinase C; ROCK, RhoA/Rho-kinase; PLC, phospholipase C; MR, minute rhythm; CaCCs, Ca^{2+}-activated Cl- channels; NCXs, Na+/Ca^{2+} exchanger; STICs, spontaneous transient inward currents; 18β-GA, 18β-glycyrrhetinic acid; ChAT, choline acetyltransferase; NPY, neuropeptide Y; SST, somatostatin; VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; nNOS, neuronal nitric oxide synthase; TKs, tachykinins; Ach, acetylcholine; NO, nitric oxide; cGMP, 3',5'-cyclic-guanosine monophosphate; CO, carbon monoxide; NT, neurotensin; PYY, peptide YY; TGR5, Takeda GPCR 5; FXR, FGF15/19-farnesoid X receptor; GLP-2, glucagon-like peptide 2; SCF, stem cell factor; AC, acute cholecystitis; AAC, acute acalculous cholec-ystitis; PGs, prostaglandins; ET, endothelin; PGE2, prostaglandin E$_2$

between meals and regulates the outflow of bile into the duodenum postprandial. The human liver normally produces at least 1,000 ml of hepatic bile per day (1). Up to 80% of hepatic bile partitions into the GB, depending on the synergy state of the GB and sphincter of Oddi (2,3). The GB undergoes structural and functional changes, as well as GB dysmotility, in numerous pathological conditions, including gallstone disease, GB polyps and acute acalculous cholecystitis (4-6). Given that GB dysmotility is so prevalent in GB disease, a comprehensive understanding of the neurons and smooth muscles responsible for GB contractile activity is critical.

GI motility patterns, including those of the GB, result from coordinated contractions of the muscular layers of the alimentary canal. Several studies found that interstitial cells of Cajal (ICCs) and platelet-derived growth factor receptor α-positive (PDGFRα⁺) cells form electrical coupling complexes with smooth muscle cells (SMCs) in the GI tract. Sanders *et al* (7) initially proposed this structure as an SMC-ICC-PDGFRα⁺ cell (SIP) syncytium. In this functional structure, ICCs act as periodic spontaneous pacemakers to generate a slow wave (SW), which conducts SMCs to drive phasic contractions (8,9). Correspondingly, PDGFRα⁺ cell excitation causes hyperpolarization of SMCs, leading to muscle relaxation (10,11). Unlike skeletal muscle, there is no classical neuromuscular junction between nerve terminals of the enteric nervous system (ENS) and GI smooth muscle (12). Enteric nerve endings expand to form numerous varicosities containing neurotransmitters (13,14). Subsequently released neurotransmitters diffuse to the adjacent SIP syncytium to regulate GI motility. Although the integrity of the morphological structure and function of SIP syncytium are important for GI physiological function, the functions of SIP syncytium are mainly derived from evaluations of specific SIP cell types.

Previously, telocytes (TCs) were considered interstitial Cajal-like cells (ICLCs) due to the similar morphology under the light microscope and immunohistochemical (IHC) features with ICCs, which were found >100 years ago and considered to be pacemakers for GI motility. Subsequently, it was demonstrated that TCs are not ICLCs, as TCs presented a distinctly different ultrastructure from ICLCs in transmission electron microscopy (TEM) images. To avoid further confusion and to give a precise identity to these cells, in 2010, Popescu and Faussone-Pellegrini (15) coined the term TCs for cells previously referred to as ICLCs. Differences in the TCs' immune phenotypes have been found to be significant in different tissues; by contrast, the ultrastructural differences of TCs are the least evident. Hence, the term TCs was proposed based on the cells' unique TEM features rather than selective immune markers. Subsequently, Vannucchi *et al* (16) clearly indicated that TCs express PDGFRα in the human GI tract. Based on these IHC data, TCs are frequently referred to as PDGFRα⁺ cells and this definition is commonly used in scientific reports. Of note, as TCs express different IHC markers in different organs and even in different tissues from the same organ, it remains controversial whether TCs and PDGFRα⁺ cells are the same cell type (17-20). However, in the gut, all cells identified as TCs were double-positive for CD34 and PDGFRα and shared identical ultrastructural features (16,21); therefore, these TCs and PDGFRα⁺ cells are the same cell type, at least in GI tract. Further research

substantiated the existence of TCs in the biliary system, including GB, extrahepatic bile duct, cystic duct, common bile duct and sphincter of Oddi (22).

Current electrophysiological studies of the GI tract are mostly focused on the stomach and intestine. The concept of SIP syncytium was also first demonstrated and proposed in the GI tract (7). Although the histological anatomy and physiological functions of the GB and the stomach or intestine are not identical, they belong to the same myogenic organs of the digestive tract and their physiological functions are both dependent on the movement of their smooth muscles. More importantly, both the expression and distribution of ICCs and TCs have also been demonstrated in myogenic organs such as the GB, ureter and uterus (23-26). Current studies on GB electrophysiology are mainly on SMCs and ICCs (22,27-33). The mechanisms of SMCs in the motor function of the GB have been most thoroughly studied. It is currently believed that ICCs in GB have a regulatory role in the motor function of the GB, but the exact mechanism of regulation remains to be clarified. The study of TCs in the GB is even more limited to histology. However, the regulation of GB motor function is important for benign GB diseases (e.g., cholelithiasis, cholecystitis, GB polyps, GB adenomyosis). In the most recent study by our group, the presence of a unique structure containing ICCs, TCs, SMCs and neurons in the GB has been proved by multiplexed IHC (Fig. 1; for methods see supplementary data). These results indicated that the four cells were in spatial proximity to each other in mouse GB. Furthermore, c-Kit and anoctamin 1 (Ano1) were used to label ICCs, CD34 and PDGFRα to label TCs, Myh11 and Acta2 to label SMCs to analyse the single-cell RNA-sequencing of normal mice (for methods see supplementary data) (34). The results also proved that there were three double-positive cell types (ICCs, TCs and SMCs) for their respective specific molecular markers and they formed their own cell clusters (Fig. 2). All of these results demonstrated that these four types of cells are present and constitute the SMC-TC-ICC-neuron (STIN) syncytium structure in the mouse GB. Based on these findings, the functional complex was proposed as an STIN syncytium (Fig. 3). The present review described various aspects of the morphology, regulation and function of STIN cells in GB and discussed pathological changes of the STIN syncytium in GB disease.

2. Morphology and distribution of STIN cells

Research of GB structure and function is primarily derived from animal studies, particularly guinea pig and mouse models. The identification of individual STIN cells is based on their morphology (Fig. 4; for methods see supplementary data) and immune phenotypes, which are summarized in Table I.

GB smooth muscle cells (GSMCs). Unlike the GI tract, the GB muscle layer only consists of a single layer of SMCs. GB muscle fibers are separated by different amounts of connective tissue and orientated in different directions (35). GSMCs are shuttle-shaped, with abundant thin (actin and calponin) and thick filaments (myosin) in the cell body. Typical binding of actin and myosin results in cross-bridges, which form
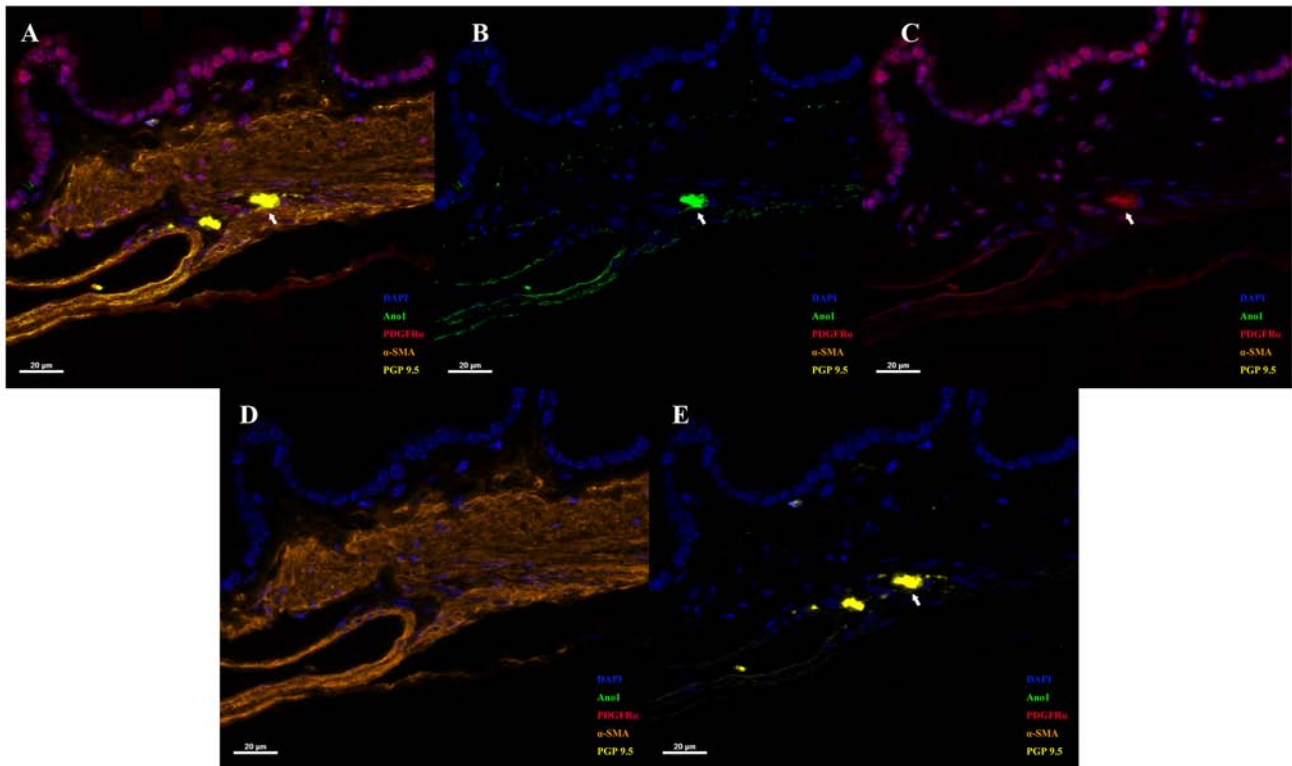
Figure 1. Full-thickness sections of mouse GBs stained by multiplexed immunohistochemistry methods to visualize STIN cells. (A) GB wall containing STIN cells (arrow). Ano1-immunopositive reactivity is displayed in green, PDGFRα-positive in red, α-SMA-positive in orange, PGP 9.5-positive in yellow and cell nuclei were counterstained with DAPI (blue). (B) GB wall containing ICCs marked with Ano1 (arrow). (C) GB wall comprising TCs marked with PDGFRα (arrow). (D) GB wall including GSMCs marked with α-SMA. (E) GB wall containing neurons marked with PGP 9.5 (arrow) (scale bars, 20 μm). STIN, SMCs-TCs-ICCs-neurons; ICCs, interstitial cells of Cajal; TCs, telocytes; GSMCs, gallbladder SMCs; SMCs, smooth muscle cells; PDGFRα, platelet-derived growth factor receptor α; Ano1, anoctamin 1; SMA, smooth muscle actin; GB, gallbladder; PGP 9.5, protein gene product 9.5.
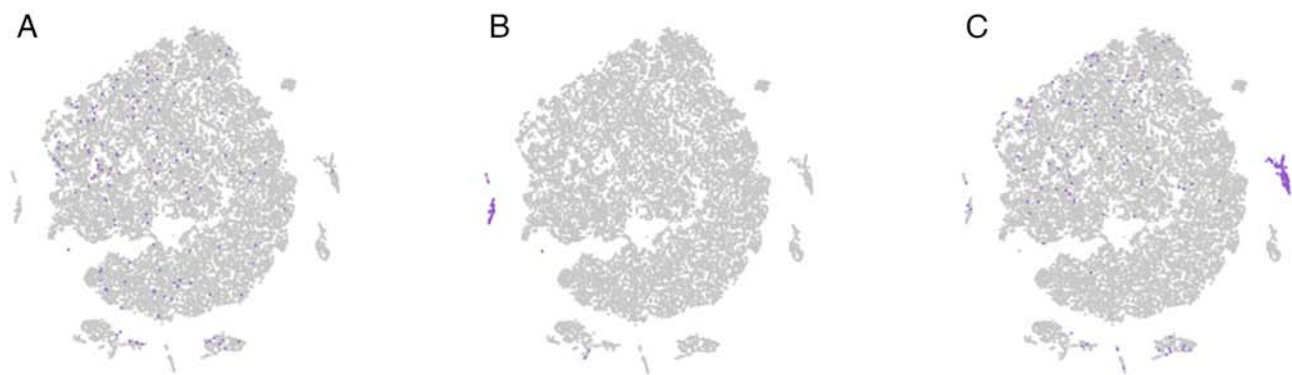

Figure 2. T-distributed stochastic neighbor embedding plot indicating the expression of known marker genes for cell types of normal mouse gallbladder. (A) Interstitial cells of Cajal, c-Kit and anoctamin 1. (B) Telocytes, platelet-derived growth factor receptor α and CD34. (C) Smooth muscle cells, Myh11 and Acta2. The raw data are from the Gene Expression Omnibus dataset GSE179524.

the basic unit of smooth muscle movement (36). α-Smooth muscle actin (α-SMA) is frequently used as a specific marker for smooth muscle (37). Another characteristic structure of GSMCs is the plasma membrane-sarcoplasmic reticulum (SR) junction, which are invaginations of the plasma membrane containing signaling molecules and ion channels (38).

ICCs. Research on GB ICCs began in the 21st century. In 2006, Sun *et al* (39) first confirmed the existence of ICCs in CD1 mouse GB by c-Kit antibody labeling in combination with methylene-blue staining. Later, ICCs were also identified in human extrahepatic bile ducts, where they are more densely aggregated than in the GB (40,41). Light microscopy indicated that ICCs are typically elongated with oval-shaped cell bodies and 1-3 long processes extending from their poles, or exhibit a triangular cell body with several slender lateral branches (42). The fusiform ICCs form a multiple connecting network that is oriented parallel to adjacent muscle fibers in the GB muscularis layer. TEM scanning revealed that ICCs possess large nuclei, a
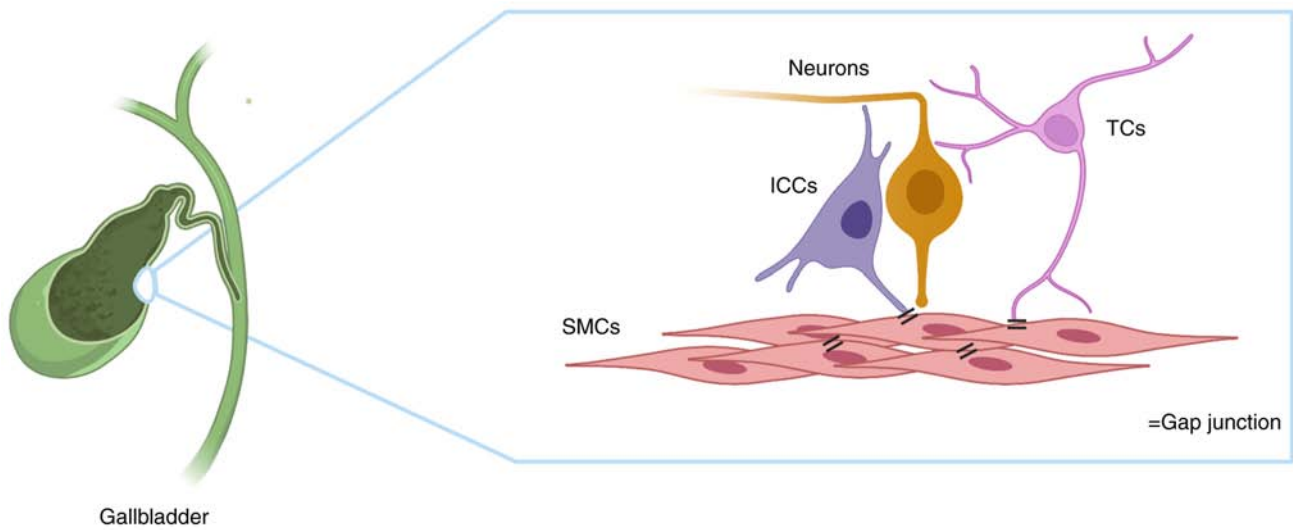
Figure 3. Cellular components of the STIN syncytium. GB neurons, ICCs and TCs are electrically coupled via gap junctions in SMCs, forming the STIN syncytium and providing regulatory control of GB function. In the muscular layer of the GB, ICCs and TCs are closely associated with the terminal processes of GB neurons and express receptors, second-messenger, neurohumoral pathways and ion channels facilitating responses to GB motor neurotransmitters. ICCs are pacemaker cells and generate electrical slow waves. TCs are responsive to purines and participate in the inhibitory neurotransmission of purinergic neurotransmitters. ICC and TCs are electrically coupled to SMCs, which may conduct slow waves to SMCs and regulate the excitability of the musculature in the gallbladder. STIN, SMCs-TCs-ICCs-neurons; ICCs, interstitial cells of Cajal; TCs, telocytes; SMCs, smooth muscle cells; GB, gallbladder.
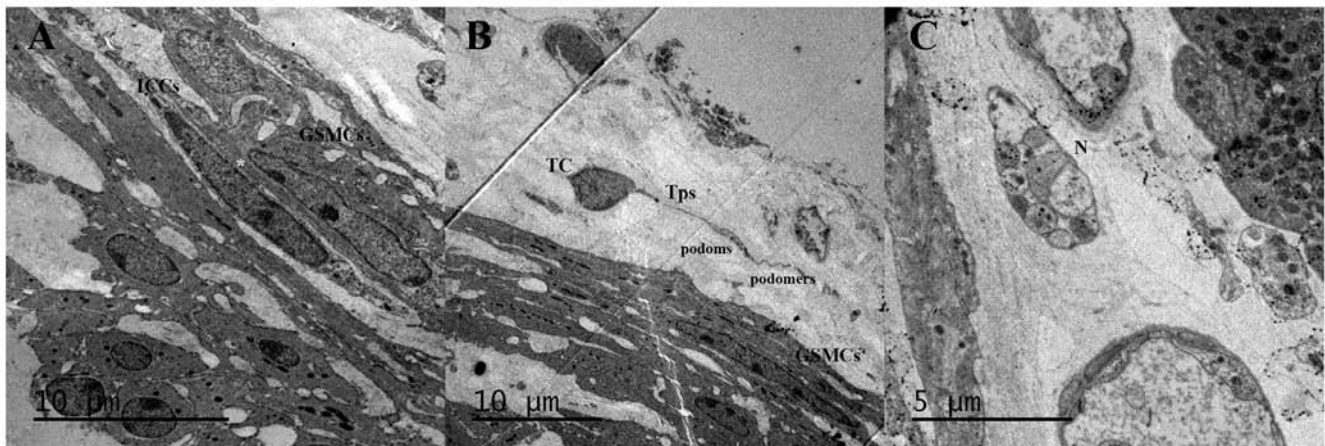

Figure 4. Transmission electron microscopy images of STIN cells in mouse GB. (A) The photographic reconstruction illustrates ICCs rich in mitochondria, smooth endoplasmic reticulum and caveolae observed in muscularis propria. The ICCs form electrical conduction structures with surrounding SMCs through gap junctions (*). (B) TCs have a small oval body, mainly occupied by the nucleus, and are thin and long; the repeatedly folded processes extend beyond the cellular body, which are called Tps. The thin segments are called podomers and the dilated regions podoms (scale bars, 10 μm). (C) The presence of typical GB nerve endings containing abundant synaptic vesicles in the muscular layer of the GB (scale bar, 5 μm). N, neuron; GSMC, GB smooth muscle cell; ICC, interstitial cells of Cajal; TC, telocytes; Tps, telopodes; STIN, SMC-TC-ICC-neurons; GB, gallbladder; SMC, smooth muscle cell.

well-developed smooth endoplasmic reticulum, abundant free perinuclear mitochondria, distinctive caveolae, free ribosomes and intermediate filaments without thick filaments, which are adjacent to SMCs and nerve endings (43). Recently, two identified genes, Ano1 and Na^+-K^+-Cl^- cotransporter (NKCC1), were found to be highly expressed in GB, representing a new and highly selective molecular marker for studying the distribution and fate of ICCs (44,45).

TCs. In 2007, Hinescu *et al* (41) first described TCs in human GB in detail. In the human adult GB, TCs are mostly placed near small vessels in the subepithelial region of the lamina propria and between smooth muscle bundles in the muscularis (46). TEM is considered the most accurate method for identifying TCs (15,26,47). In TEM images, TCs exhibit a variable tiny body with several dichotomously branched, extremely long and thin telopodes. The shape of the cytoplasm varies, including fusiform, pyriform and triangular shapes depending on the number of telopodes, which have a moniliform profile characterized by the alternation of thin tracts with dilations. Hematoxylin and eosin staining revealed long and extremely thin prolongations undetectable by light microscopy. The thin segments are called podomers, while the dilated regions are called podoms. Podoms hold functional units consisting of numerous mitochondria, endoplasmic reticulum and caveolae. CD34 and PDGFRα are considered reliable markers of TCs in the GI tract (48,49). In addition, TCs selectively express the small conductance Ca^{2+}-activated K^+ channel SK3 in the gut,

Table I. Identification of STIN cells in gallbladder.

Author(s), year	Cell types	Location	Morphology	Histochemistry	Special marker	(Refs.)
Sun et al, 2006 Pasternak et al, 2016 Lavoie et al, 2007 Gomez-Pinilla et al, 2009 Zhu et al, 2016 Pasternak et al, 2012 Burns et al, 1997 Christensen et al, 1992 Ward et al, 1990 Mikkelsen et al, 1988 Xue et al, 1993 Huang et al, 2009 Vannucchi et al, 2016	ICCs	Muscularis propria layer	Ovoid or triangular, body 1-3 cytoplasmic processes, large nuclei, abundant mitochondria, SER and characteristic caveolae, without thick filaments	Silver chromate stain, MB stain, rhodamine 123 stain, NADH diaphorase stain	c-kit (+), Ano1 (+), NKCC1 (+), CD34 (-), tryptase (-)	(39,42-46, 209-215)
Horowitz et al, 1996 Ota et al, 2021 Sugai et al, 1985 Hartshorne et al, 1998	SMCs	Smooth muscle layer	Shuttle-shaped body, numerous thin and thick filaments, plasma membrane-SR junction	Masson stain	α-SMA (+)	(36,37, 216,217)
Popescu et al, 2010 Vannucchi et al, 2013 Pieri et al, 2008 Chen et al, 2018 Cretoiu et al, 2014 Hinescu et al, 2007 Pasternak et al, 2012 Peri et al, 2013 Lu et al, 2018 Yeoh et al, 2016 Mnh et al, 1998	Telocytes	Muscular layer	Tiny variable body, hallmark Tps with podomers and podoms	MB stain, toluidine blue staining	CD34 (+), SK3 (+), PDGFRα (+)	(15,16,21,22, 26,41,46, 48-50,218)

STIN, smooth muscle cell-telocyte-interstitial cells of Cajal-neuron; SER, smooth endoplasmic reticulum; MB, methylene-blue; NADH, nicotinamide adenine dinucleotide; Ano1, anoctamin 1; NKCC1, Na$^+$-K$^+$-Cl$^-$ cotransporter; SR, sarcoplasmic reticulum; α-SMA, α-smooth muscle actin; Tps, telopodes; SK3, small conductance Ca^{2+}-activated K$^+$ channels; PDGFRα, platelet-derived growth factor receptor α.

which exhibits significant changes in functionality in the context of GI disease (50). TCs always form networks and provide mechanical support in the GI wall. However, the distribution of TCs in GB across various species remains controversial and further study is required to elucidate it.

3. GSMCs: Excitation-contraction coupling units

Depolarization of GSMCs may occur through direct effects of neurotransmitters, hormones and other bioactive regulatory substances on GSMCs, or through the influence of other STIN

cells electrically coupled to GSMCs. In general, contractions are initiated by phosphorylation of myosin light chain (MLC) 20 by Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK) or Ca^{2+}-independent myosin light chain phosphatase (MLCP) (51). Phosphorylation of MLC20 facilitates myosin binding to actin, initiating cross-bridge cycling and contraction development.

Electrical properties of GB smooth muscle (GBSM). Intracellular voltage recordings from intact guinea pig GSMCs revealed that characteristic action potentials (APs) have four distinct components: A resting membrane potential of -40 to -50 mV, a rapidly depolarizing (rarely exceeds 0 mV) and transient repolarizing spike, followed by a slowly sustained declining plateau phase, and finally complete repolarization (52).

GSMCs exhibit rhythmic spontaneous APs (0.3 to 0.4 Hz) started by Ca^{2+} entry, mainly through voltage-dependent Ca^{2+} channels (VDCCs) (52). The AP spike results from activation of L-type VDCCs in the absence of a T-type Ca^{2+} current in guinea pig GSMCs (53). The open state of L-type Ca^{2+} channels is regulated by neurotransmitters and drugs (54,55). For instance, L-type Ca^{2+} channel blockers such as nifedipine may abolish spontaneous AP and inhibit GB contraction. L-type channels are critical for proper GSMC function, providing the major source of contractile Ca^{2+}. Depolarization of I_{cat}, a spontaneously active Na^+-mediated nonselective cation channel, was indicated to maintain the resting membrane potential and increase contractility of GBSM, thus stabilizing GB tone (56).

The repolarization of APs is determined by voltage-gated K^+ (K_v) channels and ether-a-go-go-related gene (ERG) K^+ channels (57,58). Potassium reflux via K_v channels is responsible for the repolarization of APs and regulates the contraction of GBSM. These channels demonstrate relatively low sensitivity to aminopyridines but are inhibited by quinine (59). ERG, which encodes a delayed rectifier K^+ channel in GB, contributes to repolarization of both the rapid spike and plateau phase (60). ERG channel blockers prolong repolarization of the plateau phase, increasing basal contractility of GSMCs and their response to receptor activation (57).

Other potassium channels identified in GSMCs include ATP-sensitive K^+ (K_{ATP}) channels and large-conductance Ca^{2+}-activated K (BK_{Ca}) channels. Activation of the K_{ATP} channel causes prolonged hyperpolarization, reducing the frequency of GBSM APs and associated spontaneous GBSM contractions (61). The K_{ATP} channel appears to have a major role in receptor-mediated relaxation of GBSM, as it is responsible for the inhibitory effects of calcitonin gene-related peptide (CGRP) and agonists of H2 receptors for histamine (62,63). In GSMCs, localized Ca^{2+} release events from ryanodine-sensitive receptors (RyR), also called Ca^{2+} sparks, antagonize GSMC excitability by activating BK_{Ca} channels in the nearby plasma membrane (see below) (64). Spontaneous transient activation of BK_{Ca} currents causes transient membrane hyperpolarization of GSMCs that was, in part, inhibited by cholecystokinin (CCK). Additional cellular mechanisms underlying bile acid-induced GBSM relaxation *in vivo* and *in vitro* potentially include activation of BK_{Ca} channels to generate outward currents, thus counteracting contraction (65).

GSMCs also express the SK3 channel. SK3 likely physically associates with ORAI calcium release-activated calcium modulator 1 (Orai1), a plasma membrane protein, to form a signaling complex. Ca^{2+} influx through Orai1 activates SK3 to induce membrane hyperpolarization in GBSM (66). This hyperpolarizing effect of the Orai1-SK3 complex may serve to prevent excessive contraction in response to contractile agonists.

Regulation of intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. GBSM excitation-contraction (E-C) coupling is dependent on an increase in the intracellular concentration of Ca^{2+} $[Ca^{2+}]_i$, which is caused by an influx of extracellular Ca^{2+} through VDCCs and/or receptor-operated Ca^{2+} channels, as well as the release of Ca^{2+} from the SR (67). The influx of extracellular Ca^{2+} required for E-C coupling may enter cells through VDCCs, capacitative calcium entry (CCE) or nonselective cation channels (NSCCs).

The predominant class of VDCC in GSMCs is the L-type Ca^{2+} channel. As previously described, global cytosolic $[Ca^{2+}]_i$ is largely dictated by the open state probability of plasmalemmal L-type Ca^{2+} channels, while calcium entry through VDCCs is determined by the cell membrane potential (68). The depletion of intracellular calcium stores activates CCE, a Ca^{2+} entry mechanism at the plasma membrane (69). Thapsigargin, a sarcoplasmic Ca^{2+}-ATPase inhibitor, is able to prevent the accumulation of Ca^{2+} by the SR. Activation of extracellular Ca^{2+}-dependent responses and Ca^{2+} influx by thapsigargin is regarded as evidence in favor of the involvement of CCE (70). Contractile responses to Ca^{2+} re-addition following depletion of SR Ca^{2+} stores with thapsigargin strongly supports CCE as a source of activating Ca^{2+} for GBSM contraction (71). In addition, actin reorganization is proven to participate in the implementation of CCE, supporting a conformational coupling model for this process in naive SMCs (72). NSCCs in GSMCs demonstrate high selectivity for Ca^{2+} over monovalent cations, leading to activation of VDCCs mediating extracellular Ca^{2+} entry and contraction (73). Transient receptor potential (TRP) channels are a large family of NSCCs widely expressed in GSMCs (74). TRPP2 protein belongs to the TRP superfamily and is encoded by the polycystin 2 gene (75). In guinea pig GB muscle strips, knockdown of TRPP2 significantly reduced carbachol-evoked Ca^{2+} release (27). Accumulating evidence demonstrates that TRPP2 not only mediates intracellular Ca^{2+} release, but also regulates extracellular Ca^{2+} influx to enhance $[Ca^{2+}]_i$ (76-78). Furthermore, TRP protein family C (TRPC) is a candidate channel involved in CCE (28). The expression of TRPC protein depends on cytosolic Ca^{2+} levels through activation of Ca^{2+}/calmodulin-dependent kinases and cAMP-response element binding protein.

Calcium influx and release from the SR, also known as intracellular stores, are crucial for GSMC contractility, which primarily depends on increases in $[Ca^{2+}]_i$ (79). Intracellular calcium release from the SR involves the participation of two ligand-gated channel/receptor complexes [inositol 1,4,5-trisphosphate receptors (IP_3R) and RyR] and is regulated by sarcoplasmic/endoplasmic reticulum calcium ATPase (80,81). Calcium release via IP_3R is activated by IP_3, which is generated in response to numerous G-protein-coupled receptors (GPCRs) and tyrosine kinase-linked receptor

activators, including neurotransmitters, hormones and drugs. RyR mediates the rapid release of calcium from intracellular stores into the cytosol, which is essential for numerous cellular functions, including E-C coupling in muscle. Three types of rhythmic spontaneous Ca^{2+} transients were determined by laser confocal imaging of intracellular Ca^{2+} in GBSM whole-mount preparations (31,64,79). Ca^{2+} flashes reflect calcium entry associated with spontaneous APs and simultaneously occur in all GSMCs in the given bundle, although they are asynchronous among nonintersecting bundles. Ca^{2+} waves are rhythmic Ca^{2+} transients propagating within GSMCs that are asynchronous between individual muscle cells in the given bundle; apparently, these waves correspond to subthreshold depolarization of GSMCs. Both flashes and waves triggered by Ca^{2+} release from the SR occur through IP_3 receptors, which is amplified by calcium-induced calcium release (CICR) and VDCCs (82). Superimposed Ca^{2+} waves induce Ca^{2+} flashes, while the summation of spontaneous transient depolarizations results in APs. In the guinea pig GB, rapid Ca^{2+} transients occur simultaneously in all the GSMCs of a given bundle, but without synchronization between muscle bundles (38). Of note, synchronous Ca^{2+} flashes occur among smooth muscle bundles in the presence of CCK or muscarinic agonists. These findings indicate that the net tone in the GB originates from asynchronous, multifocal contractions of bundles throughout the tissue wall, while synchronous electrical rhythms occurring in all muscle bundles may contribute to GB emptying. Therefore, flashes and waves are critical in maintaining the basal tone and neurohormonal-induced stimulation of GB motility and emptying. Ca^{2+} release from intracellular stores not only induces contraction, it also induces relaxation. Ca^{2+} sparks are another type of focal, nonpropagating calcium transients caused by the coordinated opening of a cluster of RyR. In GB, Ca^{2+} sparks do not lead to any elevation in global $[Ca^{2+}]_i$. Instead, transient localized $[Ca^{2+}]_i$ elevations through opening of BK_{Ca} channels cause SMC hyperpolarization and relaxation (64).

Ca^{2+}-independent MLCP pathway. GSMC contraction is also regulated by Ca^{2+}-independent mechanisms via protein kinase C (PKC)/CPI-17 or RhoA/Rho-kinase (ROCK)-mediated pathways. The regulation of MLC phosphorylation by MLCK causes SMC contraction, whereas inhibition of MLCP may enhance the extent of MLC phosphorylation and SMC contraction and increase Ca^{2+} sensitivity, a phenomenon known as Ca^{2+} sensitization (83). In the classical PKC/CPI-17 pathway, G proteins cause activation of phospholipase C (PLC), diacylglycerol output and activation of PKC. PKC phosphorylates CPI-17, an inhibitor of MLCP activity, resulting in GBSM contraction (84,85). ROCK also regulates GSMC contraction by regulating the Ca^{2+} sensitization mechanism. Contractions induced by carbachol and CCK are mediated by GPCR muscarinic M_3 receptors and CCK_1 receptors in guinea-pig GBSM (86,87). The selective ROCK inhibitor Y-27632 significantly inhibited GBSM contractions evoked by carbachol and CCK *in vitro* (30). In human GB, Y-27632 markedly reduced 5-hydroxytryptamine, neurokinin A and KCl-induced contractions (88). The results of these studies indicate that a RhoA/ROCK-mediated pathway has a role in the regulation of GSMCs.

4. ICCs: Pacemaker of SWs

GB SWs were first recorded by Romański (89) through electromyography. However, the signal of SWs was not always observed and variable in frequency and amplitude. The minute rhythm (MR), another rhythmic activity, consisted of a series of spike potentials recurring at minute intervals (90). The MR has been proven to regularly occur in the entire ovine small intestine and GB, which is controlled by both nicotinic and muscarinic receptor subtypes (91). However, it appears improbable that the MR spike bursts significantly contribute to the enhancement of GB filling or evacuation. Thus, the role of the MR in GB may be to maintain normal tension of the GB wall during the fasting period. Loss of ICCs is associated with a lack of SW activity of GB and the GI tract (92,93). However, the relationship between MR and ICCs requires further study.

Conduction of SWs and regulation of GSMCs. ICCs have an important role in producing and propagating rhythmic electrical activity and GB motility. Isolated ICCs display spontaneous electrical rhythmicity similar to the electrical activity of intact muscles. In fact, electrical coordination between regions of SMCs must occur through the integrity of ICC networks due to the lack of ion channels to regenerate or actively propagate SWs (43,94). In GBSMs, SWs may also be recorded from SMCs due to electrical coupling with ICCs. The function of SWs is to change the membrane potential from a state of low open probability for VDCCs to depolarization, which means APs, when there is an increased probability of associated ionic channel opening (9). A Ca^{2+} imaging study by Lavoie *et al* (43) indicated that the intensity of fluo-4 fluorescence in ICCs was higher than that of the surrounding GSMCs, while rhythmic Ca^{2+} flashes were synchronized in any given GBSM bundle and associated with ICCs. More importantly, gap junction blockers may eliminate or markedly disrupt spontaneous rhythmic Ca^{2+} flashes in GBSM, but persist in ICCs, whereas the selective Kit tyrosine kinase inhibitor imatinib mesylate disrupted or abolished APs and Ca^{2+} flashes in both cell types, as well as associated GBSM contractions. These results demonstrate that the spontaneous rhythmic activity detected in GBSM, which corresponds to smooth muscle bundle contractions, is generated by specialized ICCs and not an intrinsic property of GSMCs. Taken together, ICCs conduct pacemaker SWs into neighboring GSMCs, causing membrane depolarization, opening of the VDCC, intracellular Ca^{2+} release and activation of the contractile apparatus of GB. To date, no specific 'pacing region' has been identified in the GB.

Pacemaker mechanism of ICCs. ICCs serve as pacemaker cells and express a specialized apparatus that includes Ca^{2+}-activated Cl^- channels (CaCCs), T-type voltage-dependent Ca^{2+} channels, NSCCs, NKCC1, inward rectifier K^+ channels and Na^+/Ca^{2+} exchanger (NCXs). SWs recorded from ICCs have fast upstroke depolarizations with large amplitudes and a sustained plateau potential.

SWs in ICCs are mediated by activation of Ano1 channels and NSCCs. ICC depolarization depends upon activation of CaCCs encoded by the ANO1 gene, such that loss or block

of Ano1 abolishes the electrical activity of SWs in intact smooth muscles (95). Periodic activation of Ano1 channel clusters generates spontaneous transient inward currents (STICs) and subsequently initiates coordinated activation of CaCCs that summates to cause the depolarization responses known as SWs (96). The calcium entry from RyR and IP3R of ICCs during CICR appears to be the signal coupled to activation of CaCC, as these channels are sensitive to $[Ca^{2+}]_i$ (97). Of note, research on cultured ICCs indicated that NSCCs, not CaCCs, generated the inward current responsible for SWs (95,96,98,99). This may be explained by rapid loss of Ano1 expression in cell culture and alteration of the autorhythmicity retained by ICCs compared with the pacemaker activity of cells *in situ*. Unitary potentials, which are small irregular noisy fluctuations in membrane potential, may be the primary pacemaker activity that underlies SWs. These electric events were insensitive to concentrations of niflumic acid (the inhibitor of CaCC) that blocked SWs (99). The Ca^{2+}-inhibited NSCC-activated STICs observed from isolated ICCs may be responsible for unitary potentials (95). Accordingly, NSCC may contribute to the pacemaker current and generation of electrical SWs in GI smooth muscles. T-type Ca^{2+} channels coordinate Ca^{2+} release from stores in ICCs, thus controlling the openings of Ano1 channels responsible for SW currents (100). The mechanism of SW propagation in tissues has been explored by using muscle strips and partitioned recording chambers. Reduced extracellular Ca^{2+} or antagonists of T-type Ca^{2+} channels inhibit SW upstroke depolarization velocity and propagation (101). These results suggest that SWs propagate through the ICC network by a voltage-dependent mechanism that relies on activation of T-type Ca^{2+} channels (38). ICCs have been demonstrated to express genes encoding inward rectifying K^+ channels, and this inwardly rectifying conductance contributes to the regulation of resting potentials and excitability of SMCs (102).

The plateau component of SWs was dependent on the Cl^- current through CaCCs, while the activation of Ano1 channels results in efflux of Cl^- during SWs (103). Thereby, a mechanism must exist for the recovery of Cl^- loss. IHC confirmed that NKCC1 is expressed at high levels in ICCs (104). Inhibition of NKCC1 with bumetanide and gene knockout of NKCC1 both diminished the plateau component of SWs without directly affecting Ano1 or T-type Ca^{2+} channels (45,105). In isolated GB ICCs, inhibitors of mitochondrial NKCC1 also abolished spontaneous rhythmic activity, suggesting that NKCC1 may have an important role in maintaining the Cl^- gradient supporting the driving force for the inward current mediated by Ano1 (106). Furthermore, NKCC1 may elongate the plateau phase by activation of reverse-mode NCX. NCX, an ion transport protein, extrudes Ca^{2+} in parallel with the plasma membrane ATP-driven Ca^{2+} pump (107). NCX has dynamic features in the SW cycle, in which Ca^{2+} exit helps to maintain the basal $[Ca^{2+}]_i$ between SWs and deactivate Ano1 channels at the end of the plateau; furthermore, Ca^{2+} entry sustains the activation of Ano1 channels during the plateau phase of SWs (108). The longevity of the plateau phase is related to the duration of time that NCX remains in Ca^{2+} entry mode. However, the underlying molecular mechanisms of SWs in GB ICCs remain to be further elucidated.

5. Telocytes: Purinergic inhibitory neurotransmission bridge

In the GI tract, TCs are electrically coupled with ICCs and SMCs, and in close apposition with enteric motor neuron varicosities (10). IHC studies indicated that TCs highly express gap junction genes, as well as SK3 and purinergic P2Y1 receptors (48,109,110). *In vitro*, isolated TCs respond to P1Y1 agonists by activating SK3 channels (111). Purinergic compounds, such as ATP, ADP and β-NAD, elicited large-amplitude outward potassium currents in TCs that were blocked by P2Y1 receptor antagonists and SK3 channel antagonists. This outward current causes hyperpolarization of SMCs, ultimately leading to GI relaxation. Further research suggested that P2Y1 receptors mediate purinergic inhibitory responses in GI muscles, as this relaxation reaction was absent in P2Y1-knockout mice (112). These findings indicate direct innervation of TCs by motor neurons. TCs are the primary targets for purinergic neurotransmitters in inhibitory neurotransmission. The high expression of P2Y1 and SK3 in TCs has a key role in purinergic inhibitory regulation.

SMCs also express SK3 and purinergic receptors (113). However, a previous study indicated that SMCs, stimulated directly with purine agonists, exhibit either no response or small inward currents and depolarization (114). Another study suggested that the gap junction uncoupler 18β-glycyrrhetinic acid blocked neural responses in SMCs, but not in nerve processes or TCs (115). These data indicate that the large-amplitude hyperpolarization responses elicited in GI muscles by purine neurotransmission are more likely to be mediated by TCs than SMCs. Hyperpolarization responses are conducted to SMCs via gap junctions. No evidence suggests that TCs may either generate or regenerate SWs. However, there are no electrophysiological studies on GB TCs. Thus, the role of TCs in the regulation of GB motor function requires further investigation.

6. GB neurobiology

GB relaxation and contraction are primarily myogenic, but the GB plexus has a major role in monitoring the state of the GB, in turn controlling its volume, strength of contractions and bile secretion through ENS reflexes (116,117). The innervation of GB consists of the serosal plexus, muscular plexus and mucosal plexus (118). The most prominent network is the serosal plexus with small, irregularly shaped ganglia connected by bundles of unmyelinated axons (119-121). The serosal plexus is connected to nerve bundles that parallel the extensive vascular distribution in the same layer. However, in humans, the muscular plexus is prominent and does not contain ganglia (122-124). Unlike GI neurons, all GB neurons are cholinergic and immunoreactive for choline acetyltransferase (ChAT) (118). The guinea pig is the most comprehensively studied species in this field. According to chemical coding patterns, the overall population of cholinergic neurons may be divided into two distinct subtypes (125,126): The first type (accounting for >80% of neurons) is immunoreactive for substance P, neuropeptide Y (NPY), somatostatin (SST) and orphanin FQ, and ChAT; the other one is immunoreactive for vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating

polypeptide (PACAP) and neuronal nitric oxide synthase (nNOS). In humans, most GB neurons express VIP, NPY, SST and PACAP, and also contain tachykinins (TKs) (123,127,128). Electrophysiological research of GB neurons indicates they rarely exhibit spontaneous APs and must be driven by extrinsic inputs to release neuroactive compounds onto their target cells, mostly GSMCs (129,130). ICCs and TCs are also tightly associated with excitatory and inhibitory motor neurons in the GB, and connected electrically to GSMCs. Several studies have indicated that numerous neurotransmitters and hormones may regulate GB motility (Table II).

Excitatory transmitters and hormones. GB neurons are relatively unexcitable, driven instead by vagal inputs and modulated by hormones, peptides released from sensory fibers, and inflammatory mediators (118).

CCK, an important gut hormone secreted by enteroendocrine I-cells of the upper small intestine, mainly exerts its physiological functions in GB through the activation of GPCRs identified as CCK_1 receptors. CCK_1 receptors have been identified in both GSMCs and ICCs of human and guinea pig GB and are responsible for the stimulation of contraction (131,132). Previous electrophysiological studies of the GB demonstrated that CCK has presynaptic facilitatory effects within neural ganglia to increase acetylcholine (ACh) release from vagal terminals onto GB neurons, and also stimulates vagal afferent nerve fibers in the duodenum, thus increasing stimulation of vagal preganglionic neurons (133). Furthermore, CCK induces a decrease in resistance of the sphincter of Oddi, a determinant of GB emptying (134). In brief, CCK coordinates the pressure gradient in the biliary system by promoting GB emptying and relaxing the sphincter of Oddi, ultimately facilitating bile evacuation during the feeding period.

Co-expression of TKs with ACh in GB neurons indicates that these factors may act together to promote GB emptying following afferent nerve stimulation (130). M_3 receptors are the major muscarinic receptor in GB and M_4 receptors appear to enhance carbamylcholine-induced contractility of GBSM (135). Release of ACh from neurons results in the contraction of GBSM via activation of M_3 receptors on GSMCs. Activation of M_3 receptors leads to phosphatidylinositol hydrolysis by the G protein-coupled PLC pathway and inhibits cAMP accumulation (136). In human GB, M_3 muscarinic receptors are mainly regulated by voltage-gated Ca^{2+} channels and ROCK (137). The TKs contract the guinea pig GB *in vivo* and *in vitro* by acting on NK2 receptors (138). TKs-induced muscle contraction involves activation of PKC, for which stimulation of inositol phospholipid hydrolysis was associated with the state of NK2 receptors (139).

Bradykinins and their receptors (B_1 and B_2) are potent mediators of inflammation, smooth muscle contraction and nociception. In human and guinea pig GB, bradykinin has been demonstrated to evoke a robust contraction via B_2 receptor activation (140,141). Bradykinin-induced contraction of GBSM *in vitro* relies on the synthesis of prostanoids, whose activation evokes inflammatory responses either by direct stimulation of effector cells or through the release of other mediators, including prostanoid, NO and peptide neurotransmitters. By contrast, B_1 receptors are rarely expressed in normal GB and their upregulation most probably depends on the inflammatory state of the tissue. Activation of B_1 receptors has been related to the maintenance of chronic pain and inflammation (142). Thus, the kinins system has a major role in evoking contraction in normal and, in particular, inflamed GB by stimulating both B_1 and B_2 receptors.

The physiological source of ATP in GB remains elusive and it is possible that ATP functions as a neurotransmitter (143). ATP is known to act on two different classes of P2 receptors, P2X ion channels and G-protein-coupled P2Y receptors (144). The dominant role of G protein-coupled $P2Y_4$ receptors in ATP-induced contraction has been confirmed in guinea pigs. ATP likely stimulates $P2Y_4$ receptors within GSMCs and, in turn, prostanoid production via cyclooxygenase-1, leading to increased excitability of GBSM (145). In the guinea pig, high levels of $P2X_2$ and $P2X_3$ expression are found in sensory fibers of the paravascular plexus. Double labelling IHC revealed that $P2X_2$ and $P2X_3$-immunoreactive neurons were also immunoreactive for VIP, CGRP and nNOS (146).

Inhibitory transmitters and hormones. Neurotransmitters that have an inhibitory effect on GBSM include calcitonin, CGRP, VIP, PACAP and NO. Humoral factors that relax the GB include pancreatic polypeptide (PP), SST and fibroblast growth factor (FGF)15 in mice or FGF19 in humans.

CGRP may induce concentration-dependent relaxation of GB *in vivo*, but has no effect on resting GB pressure (147). CGRP did not affect the release of CCK and the excitatory effect of CGRP was completely abolished by pretreatment with atropine. This implies that the site where CGRP activates contractile activity is on intramural cholinergic neurons rather than GSMCs. This relaxation is primarily due to the opening of K_{ATP} channels, as well as the cAMP pathway (62,148). The increased levels of NO observed when CGRP was present suggest NO is also involved in the CGRP-induced relaxation response (149). NO has been proposed to serve as a neurotransmitter in non-adrenergic non-cholinergic nerves. Synthesized by nNOS, NO stimulates soluble guanylate cyclase enzyme in GSMCs, leading to the formation of 3',5'-cyclic-guanosine monophosphate (cGMP), which mediates GB relaxation (150). Endogenous carbon monoxide (CO) produced in the GB may act as a mediator in relaxation reactions by increasing cGMP levels (151). Of note, despite persistent nNOS expression in heme oxygenase 2-knockout mice, their responses to stimulation are nearly abolished, whereas exogenous CO restored normal responses, indicating that NO does not function in the absence of CO generation (152).

VIP and PACAP are members of a VIP-secretin-glucagon superfamily of structurally related peptide hormones that exert their physiological actions through three GPCRs: PAC_1, $VPAC_1$ and $VPAC_2$ (153). VIP is thought to work as a neurotransmitter of vagus nerve terminals, which relaxes GBSM, decreases GB pressure and inhibits CCK-induced contractions (127,154,155). PACAP was able to produce both contraction and relaxation of CCK-induced GB preparations according to the resting GB tone (156). The dual effects of PACAP are likely mediated through a different type of receptor. Specifically, PACAP induces GB contraction through binding of PAC_1 receptors in unstimulated strips, while the relaxant effect of PACAP in CCK-contracted muscle strips appears to be directly mediated by GSMCs through $VPAC_2$ receptors (157).

Table II. Neuroactive compounds in STIN syncytium.

A, Excitatory compounds

Author(s), year	Neuroactive compounds	Receptors/ synthetase	Mechanisms	Effectors	(Refs.)
Yu *et al*, 1998 Schjoldager *et al*, 1989 Xu *et al*, 2008 Mawe *et al*, 1994 Behar *et al*, 1987 Cawston *et al*, 2010	CCK	CCK_1 receptors	GPCRs-PLC pathway; induction of ACh release	Facilitation of bile evacuation by coordinating the pressure gradient in the biliary system	(86,131-134, 219)
Stengel *et al*, 2002	ACh	M_2 and M_3 receptors	GPCRs-PLC pathway;	Activation of M_2 and M_3 receptors resulting in the contraction of the GB	(135-137)
Takahashi *et al*, 1994			RhoA/ROCK pathway		
Lee *et al*, 2013		M_4 receptors		M_4 receptors appear to be required for optimal functioning of M_2 and M_3 receptor	
Patacchini *et al*, 1992 Yau *et al*, 1990	TKs	NK_2 receptors	PLC-PKC pathway	Excitation GSMCs	(138,139)
O'Riordan *et al*, 2001	BKs	B_1 receptors	Receptor upregulation	Upregulation under inflammatory pathological states	(140-142)
Trevisani *et al*, 2003 Andre *et al*, 2008		B_2 receptors	COX-1	Induction of PE synthesis	
Takahashi *et al*, 1987 Bartoo *et al*, 2008	ATP	$P2Y_4$ channels	COX-1	Induction of PE synthesis	(143,145)
Greaves *et al*, 2000 Parkman *et al*, 1997	PACAP	PAC_1 receptors	PLC-PKC pathway	Excitation of resting state of the GB	(156,157)

B, Inhibitory compounds

Author(s), year	Neuroactive compounds	Receptors/ synthetase	Mechanisms	Effectors	(Refs.)
Zhang *et al*, 1994 Kline *et al*, 1997 Kline *et al*, 1994 Zhang *et al*, 1994	CGRP	CGRP receptors	cGMP-PKG pathway	Hyperpolarization of GSMCs via K_{ATP} channel; Relaxation GSMCs	(62,148, 149,220)

Table II. Continued.

B, Inhibitory compounds

Author(s), year	Neuroactive compounds	Receptors/synthetase	Mechanisms	Effectors	(Refs.)
				via dephosphory-lation of MLC; Induction of NO release of GB neurons	
Gultekin et al, 2006 Luman et al, 1998	NO	nNOS	cGMP-PKG pathway	Relaxation of GSMCs via dephosphory-lation of MLC	(150,221)
Alcón et al, 2001 Xue et al, 2000 Farrugia et al, 1998	CO	HO-2	cGMP-PKG pathway; Interaction with NO as cotransmitters	Relaxation of GSMCs via dephosphory-lation of MLC; CO may enhance nNOS catalytic activity or facilitate NO release from GB neurons	(151,152, 222)
Harmar et al, 2012 Pálvölgyi et al, 2005 Pang et al, 1998 Greaves et al, 2000 Parkman et al, 1997 Zhang et al, 2014 Morales et al, 2004 Bitar et al, 1982	VIP	VPAC$_1$ and VPAC$_2$ receptors	cAMP-PKA pathway; Interaction with nNOS	Hyperpolarization of GSMCs via K$_{ATP}$ channel; Inhibition of the CCK-induced contraction, while increasing the tension of the sphincter of Oddi	(153-157, 223-225)
Harmar et al, 2012 Pang et al, 1998 Greaves et al, 2000 Parkman et al, 1997 Morales et al, 2004	PACAP	VPAC$_2$ receptors	cAMP-PKA pathway	Hyperpolarization of GSMCs via K$_{ATP}$ channel	(153,155-157,224)
Lavoie et al, 2010 Jain et al, 2012 Yusta et al, 2017 Kliewer et al, 2015	BAs	FGF15/19	FGF15/19-FXR pathway	Partly rely on the cAMP-PKA pathway to relax GSMCs	(172-175, 226)

Table II. Continued.

B, Inhibitory compounds

Author(s), year	Neuroactive compounds	Receptors/ synthetase	Mechanisms	Effectors	(Refs.)
Choi *et al*, 2006		TGR5 receptors	cAMP-PKA pathway	Hyperpolarization of GSMCs via K_{ATP} channel	
		GLP-2 receptors	TGR5-GLP-2 pathway	Binding of TGR5 in L cells and promotion of GLP-2 release	
Vu *et al*, 2001 Maselli *et al*, 1999 Yamasaki *et al*, 1995 Kaczmarek *et al*, 2010	SST	SST receptor 2 and SST receptor 5	/	Reduction of CCK secretion as well as ACh release; Inhibition of intrinsic excitatory innervation of GB	(167,168, 227,228)
Mawe *et al*, 2001 Holzer *et al*, 2012 Chen *et al*, 1998	NPY	Y_1 and Y_2 receptors	/	Sympathetic nerves pathway	(125,161, 162)
Holzer *et al*, 2012 McGowan *et al*, 2004 Hoentjen *et al*, 2001	PYY	Y_2 receptors	/	Inhibition of vagal-cholinergic pathway	(161,163, 164)
Holzer *et al*, 2012 Hazelwood *et al*, 1993 Kojima *et al*, 2007	PP	Y_4 receptors	/	Influence on the afferent hepatic vagus	(161,165, 165)

STIN, SMC-telocyte-interstitial cells of Cajal-neuron; CCK, cholecystokinin; GPCRs, G-protein-coupled receptors; PLC, phospholipase C; ACh, acetylcholine; M, muscarinic; ROCK, Rho-kinase; TKs, tachykinins; NK, neurokinin; PKC, protein kinase C; GB, gallbladder; GSMCs, GB smooth muscle cells; BKs, bradykinins; PE, prostaglandin E; PACAP, pituitary adenylate cyclase-activating polypeptide; CGRP, calcitonin gene-related peptide; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; K_{ATP}, ATP-sensitive K^+ channel; MLC, myosin light chain; NO, nitric oxide; CO, carbon monoxide; HO, heme oxygenase; nNOS, neuronal nitric oxide synthase; VIP, vasoactive intestinal polypeptide; PKA, protein kinase A; BAs, bile acids; FGF, fibroblast growth factor; TGR5, Takeda GPCR 5; GLP, glucagon-like peptide; SST, somatostatin; NPY, neuropeptide Y; PYY, peptide YY; PP, pancreatic polypeptide.

Other gut hormones, such as the NPY family, SST and neurotensin (NT), also enhance GB relaxation (158-160). However, it remains elusive whether these hormones regulate GB tone through direct effects on ICCs, GSMCs and TCs, as there is no direct evidence that their respective specific receptors are expressed in GB. The NPY family contains biological active peptides of the gut-brain axis, including NPY, peptide YY (PYY) and PP (161). In guinea pigs, sympathetic postganglionic nerves are immunoreactive for NPY (125). These nerves likely represent the principal source of inhibitory neural input to the GB, leading to a decline of GB tone (162). PYY and PP are almost exclusively expressed in the GI tract. PYY is a GI peptide secreted from endocrine L cells localized in the distal small intestine, colon and rectum (163). PYY was able to abolish the cephalic phase of postprandial GB emptying and probably acts via vagal-dependent rather than

CCK-dependent pathways (164). PP is postprandially secreted from the pancreas, in which it is synthesized by endocrine F cells of the pancreatic islets. Similar to PP, PYY infusion results in increased volume and filling of the GB (165). Circulating PP binds to Y4 receptors in the dorsal vagal complex and affects the hepatic vagal afferent, leading to the inhibition of GB contraction and pancreatic exocrine secretion (166). SST, a peptide with potent inhibitory actions on GB contraction, enhances GB relaxation and reduces plasma excitatory gut hormone (ACh and CCK) secretion during the late postprandial phase (167). SST at a pathological concentration was able to inhibit the GB motor response to intrinsic excitatory innervation *in vitro* (168). NT, a peptide consisting of 13 amino acids, may either stimulate or inhibit GB motility, depending on the dose and species (169). NT induced a dose-dependent contraction of isolated GB of guinea pigs, and these contractile effects resulted from the excitement of cholinergic neurons in the myenteric plexus of GB (170). However, intravenous infusion of NT caused relaxation of the GB in humans (160). Of note, this contractile response was not observed *in vitro* (171).

Recently, bile acids (BAs) have been recognized as signaling molecules capable of regulating GB filling through two different mechanisms: The BAs-Takeda GPCR 5 (TGR5) pathway and the FGF15/19-farnesoid X receptor (FXR) pathway. TGR5 expression was identified in both enteroendocrine L cells and GSMCs (172,173). First, separate BAs were able to directly bind TGR5 in GSMCs, promoting GB filling. In addition, BAs in the intestinal lumen stimulated TGR5 on enteroendocrine L cells, which released glucagon-like peptide 2 (GLP-2) that subsequently activated GLP-2 receptors on GSMCs, ultimately mediating relaxation (174). BAs also activate the FXR expressed by enterocytes, thereby mediating the synthesis and release of FGF15/19 into the blood and subsequent stimulation of FGF receptors on GSMCs, inducing GB relaxation (175). Of note, activation of FXR of enteroendocrine L cells may inhibit GLP-2 release, and this effect may antagonize BA-induced relaxation of GB under certain circumstances.

7. STIN syncytium and the pathophysiology of GB diseases

Cholelithiasis. Cholelithiasis is a highly prevalent digestive system disorder with high socioeconomic costs worldwide (176). In China, the incidence of cholelithiasis is nearly 8-10% and has been gradually increasing in recent years (177). Depending on individual composition and location, gallstones contain >90% cholesterol and the remaining material is black or brown pigment stones (4).

The loss of ICCs results in GB dysmotility in patients with cholesterol or pigment stones, as well as animal models of gallstone disease (33,178). Hypercholesterolemia is an independent risk factor for cholelithiasis, as it may increase biliary cholesterol concentrations, consequently leading to bile crystallization and, ultimately, gallstone formation (179,180). More importantly, cholesterol accumulation strongly damaged the density and ultrastructure of GB ICCs by inhibiting the stem cell factor (SCF)/c-Kit pathway, and disrupted membrane receptor functions of STIN cells, particularly CCK1 receptors (181-183). Due to impaired CCK-induced emptying, the resulting GB stasis provides a microenvironment for excess

cholesterol to remain in the lumen; in turn, the elevated cholesterol content further impairs GB emptying (184). During the chronic pathogenesis of cholelithiasis, cholesterol induces an oxidative stress response with characteristic concentration dependence, resulting in inhibited proliferation and continuous apoptosis of GB ICCs (185,186). *In vitro* studies suggested that cholesterol decreases Ca^{2+} channel function and the fluidity of caveolar regions, causing sequestration of excitatory receptors to support reduced binding of agonists in affected GBSM (187,188). High cholesterol diets also significantly inhibit ROCK expression in GMSCs, leading to the promotion of gallstone formation (189). Therefore, enhancement of ROCK expression in GSMCs may be a novel strategy for the prevention and treatment of cholelithiasis.

Hydrophobic bile salts decrease GB contractility, an effect directly related to the hydrophobicity of bile salt (190,191). Hydrophobic bile salts hyperpolarize GSMCs by binding to the GPCR GPBAR1 (also known as TGR5) and activating cAMP-mediated opening of K_{ATP} channels, eventually disrupting GBSM function (172). The reduction in the number of ICCs may be a consequence of the toxicity of hydrophobic bile salts, while other bile components (such as glycocholic and taurocholic acids) may exert protective effects on ICCs (192). However, whether BAs are able to directly injure ICCs requires further study.

Patients with gallstones display abnormalities of the GB neural network. Specifically, IHC of GB with gallstones featured a significant decrease of neurons and enteric glial cells compared with that of GB without gallstones, while calretinin-positive neurons were not different between the two groups of patients (193). Calretinin has been identified as a marker of Dogiel type II gut neurons, which appear to behave as mechanosensors. Thus, these findings support the hypothesis that GB wall mechanics remain intact in patients with or without gallstones, whereas GB motility is impaired.

Acute cholecystitis. Gallstones are responsible for 90-95% of cases of acute cholecystitis (AC), while ~5-10% of patients exhibit acute acalculous cholecystitis (5,194). The pathogenesis of AC is complex and multifactorial, but GB dysmotility is the most critical pathogenic factor, as it may cause GB ischemia, cholestasis and secondary bacterial infection.

Inflammation induces alterations of Ca^{2+} sensitization observed in AC by desensitizing Ca^{2+} pools and impairing the functional status of plasma membrane Ca^{2+} channels (195). Inflammation also reduces the expression of contractile proteins, such as F-actin in GSMCs, which may be responsible for the observed reduction in sensitivity of E-C coupling (195). Inhibition of MLCP mediated by the RhoA/ROCK pathway may also be responsible for the impairment of the contractile response (84). Hydrophobic bile salts may enhance inflammatory processes, as they may diffuse through the mucosa and affect the generation of reactive oxygen species (ROS) by GBSM, either by direct action on GSMCs or increasing numbers of inflammatory cells in the GB wall (196).

Like other inflammatory processes, AC involves the release of inflammatory factors, including prostaglandins (PGs), ROS, histamine and endothelin (ET). Early studies of AC patients demonstrated that both the mucosa and muscularis of GB produce high levels of PGE_2 and the severity of inflammation

was associated with the concentration of PGE_2 (197). Symptoms of AC are significantly reduced during the first 24 h by the cyclooxygenase inhibitor indomethacin (198). Furthermore, PGE_2 has been indicated to hyperpolarize GB neurons, thereby inhibiting neurogenic contractions of GB (199). Normally, ROS produced during oxidative metabolism is cleared by antioxidant mechanisms, yet oxygen-derived free radical production may exceed the capability of scavengers, resulting in ROS accumulation and pathogenic effects during inflammation. Furthermore, during inflammation, excessive production of NO through inducible NOS with concurrent ROS production increases H_2O_2 formation (200,201). Exogenous H_2O_2 causes GBSM contraction and impairs GB responses to agonists of membrane-dependent receptors, thus inducing GBSM impairment (201,202). Histamine is released from mast cells, which are abundant in the GB wall. In GSMCs, histamine performs diametrically opposed functions through H_1 and H_2 receptors. Activation of H_1 receptors depolarizes GSMCs, whereas activation of H_2 receptors causes hyperpolarization via K_{ATP} channels (63,203). However, the net effect of histamine in GB is normally contraction (204). Although the role of histamine in AC is not fully understood, it is possible that AC is associated with increased mast cell infiltration and degranulation. ETs are bioactive peptides produced by GB epithelial cells, which have a crucial role in the early inflammatory process of AC. GB tissue ET levels are elevated, which is accompanied by an increase in GB tone (205). This pathological change precedes any histological evidence of GB inflammation. ET likely exerts an autocrine/paracrine role in the human GB via ET-a and ET-b receptors of GBSM (206). Pretreatment of the GB with an ET antagonist abrogated the development of AC.

In addition, decreased GB motility in AC results from the effects of neutrophils on the development and function of the ICCs network via depression of SCF/c-Kit expression (207). Upon coculture with neutrophils *in vitro*, the intracellular calcium transient of ICCs was less sensitive to contraction agonists and inhibitors (208). A study of human GB strips from AC suggested that overexpression of B_1 receptors by GSMCs may contribute to the typical symptoms that underline biliary colic during the cholecystitis state (142).

8. Conclusions

In summary, regulation of the membrane potential is complex, as GSMCs are electrically coupled to ICCs and TCs. Activation of conductance in any STIN cell affects the excitability of the syncytium. Individual STIN cells express intrinsic electrophysiological mechanisms and a variety of receptors for neurotransmitters, hormones, paracrine substances and inflammatory mediators. Similar to other GI SMCs, GSMCs rely on the formation of cross-bridges between actin and myosin for the development of force to empty the GB. The onset of GSMC depolarization requires SWs generated and propagated by GB ICCs. TCs (also known as PDGFRα+ cells) exert an inhibitory effect on the excitability of SMCs through SK3 channels in the GI tract. However, the specific role of TCs in GB has yet to be studied and is a potential topic for future electrophysiological studies of GB. Therefore, the integrated output of the STIN syncytium sets the transient excitability of GSMCs. The primary risk factor for benign GB disease is GB dysmotility. Loss and dysfunction of

STIN cells have been observed in patients and animal models with cholelithiasis and cholecystitis, suggesting that impairment of the STIN syncytium may be a critical pathogenic factor in benign GB disease. However, to date, there remains a lack of breakthroughs in the study of STIN syncytium. Thus, further research to better understand the pharmacology and physiology of the STIN syncytium is required.

Availability of data and materials

The raw single-cell RNA-sequencing data that were used to generate Fig. 2 may be obtained at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE179524.

Authors' contributions

FD and QH drafted the manuscript; MJ, RG, LL and FC prepared the figures and tables; YW and ZC critically revised the manuscript; HH and GZ conceived the review. HH and GZ checked and confirmed the authenticity of the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

References

1. Boyer J: Bile formation and secretion. Compr Physiol 3: 1035-1078, 2013.
2. Lanzini A, Jazrawi RP and Northfield TC: Simultaneous quantitative measurements of absolute gallbladder storage and emptying during fasting and eating in humans. Gastroenterology 92: 852-861, 1987.
3. Torsoli A, Corazziari E, Habib FI and Cicala M: Pressure relationships within the human bile tract. Normal and abnormal physiology. Scand J Gastroenterol Suppl 175: 52-57, 1990.

4. Lammert F, Gurusamy K, Ko CW, Miquel JF, Méndez-Sánchez N, Portincasa P, van Erpecum KJ, van Laarhoven CJ and Wang DQ: Gallstones. Nat Rev Dis Primers 2: 16024, 2016.
5. Gallaher J and Charles A: Acute cholecystitis: A review. JAMA 327: 965-975, 2022.
6. Bronkhorst MWGA, Terpstra V and Bouwman LH: Polyp in the gallbladder. Gastroenterology 141: e3-e4, 2011.
7. Sanders KM, Koh S, Ro S and Ward SM: Regulation of gastrointestinal motility-insights from smooth muscle biology. Nat Rev Gastroenterol Hepatol 9: 633-645, 2012.
8. Huizinga JD, Zarate N and Farrugia G: Physiology, injury, and recovery of interstitial cells of Cajal: basic and clinical science. Gastroenterology 137: 1548-1556, 2009.
9. Sanders KM, Koh SD and Ward SM: Interstitial cells of cajal as pacemakers in the gastrointestinal tract. Annu Rev Physiol 68: 307-343, 2006.
10. Horiguchi K and Komuro T: Ultrastructural observations of fibroblast-like cells forming gap junctions in the W/W(nu) mouse small intestine. J Auton Nerv Syst 80: 142-147, 2000.
11. Klemm MF and Lang RJ: Distribution of Ca^{2+}-activated K^+ channel (SK2 and SK3) immunoreactivity in intestinal smooth muscles of the guinea-pig. Clin Exp Pharmacol Physiol 29: 18-25, 2002.
12. Burnstock G: Autonomic neurotransmitters and trophic factors. J Auton Nerv Syst 7: 213-217, 1983.
13. Spencer NJ and Hu H: Enteric nervous system: Sensory transduction, neural circuits and gastrointestinal motility. Nat Rev Gastroenterol Hepatol 17: 338-351, 2020.
14. Obermayr F, Hotta R, Enomoto H and Young HM: Development and developmental disorders of the enteric nervous system. Nat Rev Gastroenterol Hepatol 10: 43-57, 2013.
15. Popescu LM and Faussone-Pellegrini MS: TELOCYTES-a case of serendipity: The winding way from interstitial cells of Cajal (ICC), via interstitial Cajal-like cells (ICLC) to TELOCYTES. J Cell Mol Med 14: 729-740, 2010.
16. Vannucchi MG, Traini C, Manetti M, Ibba-Manneschi L and Faussone-Pellegrini MS: Telocytes express PDGFRα in the human gastrointestinal tract. J Cell Mol Med 17: 1099-1108, 2013.
17. Rasmussen H, Rumessen JJ, Hansen A, Smedts F and Horn T: Ultrastructure of Cajal-like interstitial cells in the human detrusor. Cell Tissue Res 335: 517-527, 2009.
18. Suciu L, Popescu LM, Gherghiceanu M, Regalia T, Nicolescu MI, Hinescu ME and Faussone-Pellegrini MS: Telocytes in human term placenta: Morphology and phenotype. Cells Tissues Organs 192: 325-339, 2010.
19. Vannucchi MG, Traini C, Guasti D, Del Popolo G and Faussone-Pellegrini MS: Telocytes subtypes in human urinary bladder. J Cell Mol Med 18: 2000-2008, 2014.
20. Suciu LC, Popescu BO, Kostin S and Popescu LM: Platelet-derived growth factor receptor-β-positive telocytes in skeletal muscle interstitium. J Cell Mol Med 16: 701-707, 2012.
21. Pieri L, Vannucchi MG and Faussone-Pellegrini MS: Histochemical and ultrastructural characteristics of an interstitial cell type different from ICC and resident in the muscle coat of human gut. J Cell Mol Med 12: 1944-1955, 2008.
22. Chen L and Yu B: Telocytes and interstitial cells of Cajal in the biliary system. J Cell Mol Med 22: 3323-3329, 2018.
23. Vannucchi MG: The telocytes: Ten years after their introduction in the scientific literature. An update on their morphology, distribution, and potential roles in the gut. Int J Mol Sci 21: 4478, 2020.
24. Wang J, Jin M, Ma WH, Zhu Z and Wang X: The history of telocyte discovery and understanding. Adv Exp Med Biol 913: 1-21, 2016.
25. Vannucchi MG and Faussone-Pellegrini MS: The telocyte subtypes. Adv Exp Med Biol 913: 115-126, 2016.
26. Cretoiu SM and Popescu LM: Telocytes revisited. Biomol Concepts 5: 353-369, 2014.
27. Zhong X, Fu J, Song K, Xue N, Gong R, Sun D, Luo H, He W, Pan X, Shen B and Du J: The role of TRPP2 in agonist-induced gallbladder smooth muscle contraction. Sci China Life Sci 59: 409-416, 2016.
28. Morales S, Diez A, Puyet A, Camello PJ, Camello-Almaraz C, Bautista JM and Pozo MJ: Calcium controls smooth muscle TRPC gene transcription via the CaMK/calcineurin-dependent pathways. Am J Physiol Cell Physiol 292: C553-C563, 2007.
29. McCarron JG, Olson ML, Rainbow RD, MacMillan D and Chalmers S: Ins(1,4,5)P3 receptor regulation during 'quantal' Ca^{2+} release in smooth muscle. Trends Pharmacol Sci 28: 271-279, 2007.
30. Quinn T, Feighery R and Baird AW: Role of Rho-kinase in guinea-pig gallbladder smooth muscle contraction. Eur J Pharmacol 534: 210-217, 2006.
31. Balemba OB, Heppner TJ, Bonev AD, Nelson MT and Mawe GM: Calcium waves in intact guinea pig gallbladder smooth muscle cells. Am J Physiol Gastrointest Liver Physiol 291: G717-G727, 2006.
32. Tan YY, Ji ZL, Zhao G, Jiang JR, Wang D and Wang JM: Decreased SCF/c-kit signaling pathway contributes to loss of interstitial cells of Cajal in gallstone disease. Int J Clin Exp Med 7: 4099-4106, 2014.
33. Pasternak A, Gil K, Matyja A, Gajda M, Sztefko K, Walocha JA, Kulig J and Thor P: Loss of gallbladder interstitial Cajal-like cells in patients with cholelithiasis. Neurogastroenterol Motil 25: e17-e24, 2013.
34. Liang J, Shao W, Liu Q, Lu Q, Gu A and Jiang Z: Single cell RNA-sequencing reveals a murine gallbladder cell transcriptome atlas during the process of cholesterol gallstone formation. Front Cell Dev Biol 9: 714271, 2021.
35. Cai WQ and Gabella G: The musculature of the gall bladder and biliary pathways in the guinea-pig. J Anat 136: 237-250, 1983.
36. Horowitz A, Menice CB, Laporte R and Morgan KG: Mechanisms of smooth muscle contraction. Physiol Rev 76: 967-1003, 1996.
37. Ota N, Hirose H, Kato H, Maeda H and Shiojiri N: Immunohistological analysis on distribution of smooth muscle tissues in livers of various vertebrates with attention to different liver architectures. Ann Anat 233: 151594, 2021.
38. Sanders KM: Chapter 1-Nerves, smooth muscle cells and interstitial cells in the GI tract: Molecular and cellular interactions. Clinical and Basic Neurogastroenterology and Motility, pp3-16, 2020.
39. Sun X, Yu B, Xu L, Dong W and Luo H: Interstitial cells of Cajal in the murine gallbladder. Scand J Gastroenterol 41: 1218-1226, 2006.
40. Ahmadi O, Nicholson Mde L, Gould ML, Mitchell A and Stringer MD: Interstitial cells of Cajal are present in human extrahepatic bile ducts. J Gastroenterol Hepatol 25: 277-285, 2010.
41. Hinescu ME, Ardeleanu C, Gherghiceanu M and Popescu LM: Interstitial Cajal-like cells in human gallbladder. J Mol Histol 38: 275-284, 2007.
42. Pasternak A, Szura M, Gil K and Matyja A: Interstitial cells of Cajal-systematic review. Folia Morphol (Warsz) 75: 281-286, 2016.
43. Lavoie B, Balemba OB, Nelson MT, Ward SM and Mawe GM: Morphological and physiological evidence for interstitial cell of Cajal-like cells in the guinea pig gallbladder. J Physiol 579: 487-501, 2007.
44. Gomez-Pinilla PJ, Gibbons SJ, Bardsley MR, Lorincz A, Pozo MJ, Pasricha PJ, Van de Rijn M, West RB, Sarr MG, Kendrick ML, et al: Ano1 is a selective marker of interstitial cells of Cajal in the human and mouse gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 296: G1370-G1381, 2009.
45. Zhu MH, Sung TS, Kurahashi M, O'Kane LE, O'Driscoll K, Koh SD and Sanders KM: Na^+-K^+-Cl- cotransporter (NKCC) maintains the chloride gradient to sustain pacemaker activity in interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol 311: G1037-G1046, 2016.
46. Pasternak A, Gajda M, Gil K, Matyja A, Tomaszewski KA, Walocha JA, Kulig J and Thor P: Evidence of interstitial Cajal-like cells in human gallbladder. Folia Histochem Cytobiol 50: 581-585, 2012.
47. Cantarero I, Luesma MJ, Alvarez-Dotu JM, Muñoz E and Junquera C: Transmission electron microscopy as key technique for the characterization of telocytes. Curr Stem Cell Res 11: 410-414, 2016.
48. Peri LE, Sanders KM and Mutafova-Yambolieva VN: Differential expression of genes related to purinergic signaling in smooth muscle cells, PDGFRα-positive cells, and interstitial cells of Cajal in the murine colon. Neurogastroenterol Motil 25: e609-e620, 2013.
49. Lu C, Huang X, Lu HL, Liu SH, Zang JY, Li YJ, Chen J and Xu WX: Different distributions of interstitial cells of Cajal and platelet-derived growth factor receptor-α positive cells in colonic smooth muscle cell/interstitial cell of Cajal/platelet-derived growth factor receptor-α positive cell syncytium in mice. World J Gastroenterol 24: 4989-5004, 2018.
50. Yeoh JW, Corrias A and Buist ML: A mechanistic model of a PDGFRα(+) cell. J Theor Biol 408: 127-136, 2016.

51. Bolton TB, Prestwich SA, Zholos AV and Gordienko DV: Excitation-contraction coupling in gastrointestinal and other smooth muscles. Annu Rev Physiol 61: 85-115, 1999.

52. Zhang L, Bonev AD, Nelson MT and Mawe GM: Ionic basis of the action potential of guinea pig gallbladder smooth muscle cells. Am J Physiol 265: C1552-C1561, 1993.

53. Shimada T: Voltage-dependent calcium channel current in isolated gallbladder smooth muscle cells of guinea pig. Am J Physiol 264: G1066-G1076, 1993.

54. Wu Z and Shen W: Progesterone inhibits L-type calcium currents in gallbladder smooth muscle cells. J Gastroenterol Hepatol 25: 1838-1843, 2010.

55. Wu ZX, Yu BP, Xia H and Xu L: Emodin increases Ca(2+) influx through L-type Ca(2+) channel in guinea pig gallbladder smooth muscle. Eur J Pharmacol 595: 95-99, 2008.

56. Petkov GV, Balemba OB, Nelson MT and Mawe GM: Identification of a spontaneously active, Na⁺-permeable channel in guinea pig gallbladder smooth muscle. Am J Physiol Gastrointest Liver Physiol 289: G501-G507, 2005.

57. Parr E, Pozo MJ, Horowitz B, Nelson MT and Mawe GM: ERG K⁺ channels modulate the electrical and contractile activities of gallbladder smooth muscle. Am J Physiol Gastrointest Liver Physiol 284: G392-G398, 2003.

58. Wu ZX, Yu BP, Xu L and Xia H: Emodin inhibits voltage-dependent potassium current in guinea pig gallbladder smooth muscle. Basic Clin Pharmacol Toxicol 105: 167-172, 2009.

59. Jaggar JH, Mawe GM and Nelson MT: Voltage-dependent K⁺ currents in smooth muscle cells from mouse gallbladder. Am J Physiol 274: G687-G693, 1998.

60. Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y and Hill AP: hERG K(+) channels: structure, function, and clinical significance. Physiol Rev 92: 1393-1478, 2012.

61. Ashcroft SJ and Ashcroft FM: Properties and functions of ATP-sensitive K-channels. Cell Signal 2: 197-214, 1990.

62. Zhang L, Bonev AD, Nelson MT and Mawe GM: Activation of ATP-sensitive potassium currents in guinea-pig gall-bladder smooth muscle by the neuropeptide CGRP. J Physiol 478: 483-491, 1994.

63. Hemming JM, Guarraci FA, Firth TA, Jennings LJ, Nelson MT and Mawe GM: Actions of histamine on muscle and ganglia of the guinea pig gallbladder. Am J Physiol Gastrointest Liver Physiol 279: G622-G630, 2000.

64. Pozo MJ, Pérez GJ, Nelson MT and Mawe GM: Ca(2+) sparks and BK currents in gallbladder myocytes: Role in CCK-induced response. Am J Physiol Gastrointest Liver Physiol 282: G165-G174, 2002.

65. Dopico AM, Walsh JV Jr and Singer JJ: Natural bile acids and synthetic analogues modulate large conductance Ca²⁺-activated K⁺ (BKCa) channel activity in smooth muscle cells. J Gen Physiol 119: 251-273, 2002.

66. Song K, Zhong XG, Xia XM, Huang JH, Fan YF, Yuan RX, Xue NR, Du J, Han WX, Xu AM and Shen B: Orai1 forms a signal complex with SK3 channel in gallbladder smooth muscle. Biochem Biophys Res Commun 466: 456-462, 2015.

67. Kuo IY and Ehrlich BE: Signaling in muscle contraction. Cold Spring Harb Perspect Biol 7: a006023, 2015.

68. Wellman GC and Nelson MT: Signaling between SR and plasmalemma in smooth muscle: Sparks and the activation of Ca²⁺-sensitive ion channels. Cell Calcium 34: 211-229, 2003.

69. Putney JW: Capacitative calcium entry: From concept to molecules. Immunol Rev 231: 10-22, 2009.

70. Berridge MJ: Capacitative calcium entry. Biochem J 312: 1-11, 1995.

71. Quinn T, Molloy M, Smyth A and Baird AW: Capacitative calcium entry in guinea pig gallbladder smooth muscle in vitro. Life Sci 74: 1659-1669, 2004.

72. Morales S, Camello PJ, Rosado JA, Mawe GM and Pozo MJ: Disruption of the filamentous actin cytoskeleton is necessary for the activation of capacitative calcium entry in naive smooth muscle cells. Cell Signal 17: 635-645, 2005.

73. Albert AP and Large WA: Store-operated Ca²⁺-permeable non-selective cation channels in smooth muscle cells. Cell Calcium 33: 345-356, 2003.

74. Pan Z, Yang H and Reinach PS: Transient receptor potential (TRP) gene superfamily encoding cation channels. Hum Genomics 5: 108-116, 2011.

75. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, *et al*: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 272: 1339-1342, 1996.

76. González-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA and Cantiello HF: Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca²⁺-permeable nonselective cation channel. Proc Natl Acad Sci USA 98: 1182-1187, 2001.

77. Zhao R, Zhou M, Li J, Wang X, Su K, Hu J, Ye Y, Zhu J, Zhang G, Wang K, *et al*: Increased TRPP2 expression in vascular smooth muscle cells from high-salt intake hypertensive rats: The crucial role in vascular dysfunction. Mol Nutr Food Res 59: 365-372, 2015.

78. Spirli C, Locatelli L, Fiorotto R, Morell CM, Fabris L, Pozzan T and Strazzabosco M: Altered store operated calcium entry increases cyclic 3',5'-adenosine monophosphate production and extracellular signal-regulated kinases 1 and 2 phosphorylation in polycystin-2-defective cholangiocytes. Hepatology 55: 856-868, 2012.

79. Balemba OB, Salter MJ, Heppner TJ, Bonev AD, Nelson MT and Mawe GM: Spontaneous electrical rhythmicity and the role of the sarcoplasmic reticulum in the excitability of guinea pig gallbladder smooth muscle cells. Am J Physiol Gastrointest Liver Physiol 290: G655-G664, 2006.

80. Ehrlich BE and Watras J: Inositol 1,4,5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum. Nature 336: 583-586, 1988.

81. Xu L, Lai FA, Cohn A, Etter E, Guerrero A, Fay FS and Meissner G: Evidence for a Ca(2+)-gated ryanodine-sensitive Ca²⁺ release channel in visceral smooth muscle. Proc Natl Acad Sci USA 91: 3294-3298, 1994.

82. McCarron JG, Bradley KN, MacMillan D and Muir TC: Sarcolemma agonist-induced interactions between InsP3 and ryanodine receptors in Ca²⁺ oscillations and waves in smooth muscle. Biochem Soc Trans 31: 920-924, 2003.

83. Camello-Almaraz C, Macias B, Gomez-Pinilla PJ, Alcon S, Martin-Cano FE, Baba A, Matsuda T, Camello PJ and Pozo MJ: Developmental changes in Ca²⁺ homeostasis and contractility in gallbladder smooth muscle. Am J Physiol Cell Physiol 296: C783-C791, 2009.

84. Somlyo AP and Somlyo AV: Ca²⁺ sensitivity of smooth muscle and nonmuscle myosin II: Modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev 83: 1325-1358, 2003.

85. Louie DS: Cholecystokinin-stimulated intracellular signal transduction pathways. J Nutr 124 (8 Suppl): 1315S-1320S, 1994.

86. Yu P, Chen Q, Xiao Z, Harnett K, Biancani P and Behar J: Signal transduction pathways mediating CCK-induced gallbladder muscle contraction. Am J Physiol 275: G203-G211, 1998.

87. Parkman HP, Pagano AP and Ryan JP: Subtypes of muscarinic receptors regulating gallbladder cholinergic contractions. Am J Physiol 276: G1243-G1250, 1999.

88. Büyükafşar K, Akça T, Nalan Tiftik R, Sahan-Firat S and Aydin S: Contribution of Rho-kinase in human gallbladder contractions. Eur J Pharmacol 540: 162-167, 2006.

89. Romański KW: Ovine model for clear-cut study on the role of cholecystokinin in antral, small intestinal and gallbladder motility. Pol J Pharmacol 56: 247-256, 2004.

90. Fleckenstein P, Bueno L, Fioramonti J and Ruckebusch Y: Minute rhythm of electrical spike bursts of the small intestine in different species. Am J Physiol 242: G654-G659, 1982.

91. Romański KW: Characteristics and cholinergic control of the 'minute rhythm' in ovine antrum, small bowel and gallbladder. J Vet Med A Physiol Pathol Clin Med 49: 313-320, 2002.

92. Klein S, Seidler B, Kettenberger A, Sibaev A, Rohn M, Feil R, Allescher HD, Vanderwinden JM, Hofmann F, Schemann M, *et al*: Interstitial cells of Cajal integrate excitatory and inhibitory neurotransmission with intestinal slow-wave activity. Nat Commun 4: 1630, 2013.

93. Fan Y, Wu S, Fu B, Weng C and Wang X: The role of interstitial Cajal-like cells in the formation of cholesterol stones in guinea pig gallbladder. Hepatol Int 9: 612-620, 2015.

94. Sanders KM, Ward SM and Koh SD: Interstitial cells: Regulators of smooth muscle function. Physiol Rev 94: 859-907, 2014.

95. Hwang SJ, Blair PJ, Britton FC, O'Driscoll KE, Hennig G, Bayguinov YR, Rock JR, Harfe BD, Sanders KM and Ward SM: Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles. J Physiol 587: 4887-4904, 2009.

96. Zhu MH, Kim TW, Ro S, Yan W, Ward SM, Koh SD and Sanders KM: A Ca(2+)-activated Cl(-) conductance in interstitial cells of Cajal linked to slow wave currents and pacemaker activity. J Physiol 587: 4905-4918, 2009.

97. Pappas A and Wellman GC: Setting the pace for GI motility: Ryanodine receptors and IP3 receptors within interstitial cells of Cajal. Focus on 'intracellular Ca^{2+} release from endoplasmic reticulum regulates slow wave currents and pacemaker activity of interstitial cells of Cajal'. Am J Physiol Cell Physiol 308: C606-C607, 2015.

98. Koh SD, Sanders KM and Ward SM: Spontaneous electrical rhythmicity in cultured interstitial cells of cajal from the murine small intestine. J Physiol 513: 203-213, 1998.

99. Koh SD, Jun JY, Kim TW and Sanders KM: A Ca(2+)-inhibited non-selective cation conductance contributes to pacemaker currents in mouse interstitial cell of Cajal. J Physiol 540: 803-814, 2002.

100. Baker SA, Leigh WA, Del Valle G, De Yturriaga IF, Ward SM, Cobine CA, Drumm BT and Sanders KM: Ca^{2+} signaling driving pacemaker activity in submucosal interstitial cells of Cajal in the murine colon. Elife 10: e64099, 2021.

101. Sanders KM: Spontaneous electrical activity and rhythmicity in gastrointestinal smooth muscles. Adv Exp Med Biol 1124: 3-46, 2019.

102. Huang X, Lee SH, Lu H, Sanders KM and Koh SD: Molecular and functional characterization of inwardly rectifying K$^+$ currents in murine proximal colon. J Physiol 596: 379-391, 2018.

103. Kito Y, Mitsui R, Ward SM and Sanders KM: Characterization of slow waves generated by myenteric interstitial cells of Cajal of the rabbit small intestine. Am J Physiol Gastrointest Liver Physiol 308: G378-G388, 2015.

104. Youm JB, Zheng H, Koh SD and Sanders KM: Na-K-2Cl cotransporter and store-operated Ca^{2+} entry in pacemaking by interstitial cells of Cajal. Biophys J 117: 767-779, 2019.

105. Wouters M, De Laet A, Donck LV, Delpire E, van Bogaert PP, Timmermans JP, de Kerchove d'Exaerde A, Smans K and Vanderwinden JM: Subtractive hybridization unravels a role for the ion cotransporter NKCC1 in the murine intestinal pacemaker. Am J Physiol Gastrointest Liver Physiol 290: G1219-G1227, 2006.

106. Balemba OB, Bartoo AC, Nelson MT and Mawe GM: Role of mitochondria in spontaneous rhythmic activity and intracellular calcium waves in the guinea pig gallbladder smooth muscle. Am J Physiol Gastrointest Liver Physiol 294: G467-G476, 2008.

107. Blaustein MP and Lederer WJ: Sodium/calcium exchange: Its physiological implications. Physiol Rev 79: 763-854, 1999.

108. Zheng H, Drumm BT, Zhu MH, Xie Y, O'Driscoll KE, Baker SA, Perrino BA, Koh SD and Sanders KM: Na(+)/Ca(2 +) exchange and pacemaker activity of interstitial cells of Cajal. Front Physiol 11: 230, 2020.

109. Vanderwinden JM, Rumessen JJ, de Kerchove d'Exaerde A Jr, Gillard K, Panthier JJ, de Laet MH and Schiffmann SN: Kit-negative fibroblast-like cells expressing SK3, a Ca^{2+}-activated K+ channel, in the gut musculature in health and disease. Cell Tissue Res 310: 349-358, 2002.

110. Gallego D, Hernández P, Clavé P and Jiménez M: P2Y1 receptors mediate inhibitory purinergic neuromuscular transmission in the human colon. Am J Physiol Gastrointest Liver Physiol 291: G584-G594, 2006.

111. Kurahashi M, Zheng H, Dwyer L, Ward SM, Koh SD and Sanders KM: A functional role for the 'fibroblast-like cells' in gastrointestinal smooth muscles. J Physiol 589: 697-710, 2011.

112. Gallego D, Gil V, Martínez-Cutillas M, Mañé N, Martín MT and Jiménez M: Purinergic neuromuscular transmission is absent in the colon of P2Y(1) knocked out mice. J Physiol 590: 1943-1956, 2012.

113. Vogalis F and Goyal RK: Activation of small conductance Ca(2+)-dependent K+ channels by purinergic agonists in smooth muscle cells of the mouse ileum. J Physiol 502: 497-508, 1997.

114. Kurahashi M, Mutafova-Yambolieva V, Koh SD and Sanders KM: Platelet-derived growth factor receptor-α-positive cells and not smooth muscle cells mediate purinergic hyperpolarization in murine colonic muscles. Am J Physiol Cell Physiol 307: C561-C570, 2014.

115. Baker SA, Hennig GW, Ward SM and Sanders KM: Temporal sequence of activation of cells involved in purinergic neurotransmission in the colon. J Physiol 593: 1945-1963, 2015.

116. Furness J: The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 9: 286-294, 2012.

117. Furness JB, Callaghan BP, Rivera LR and Cho HJ: The enteric nervous system and gastrointestinal innervation: integrated local and central control. Adv Exp Med Biol 817: 39-71, 2014.

118. Balemba OB, Salter MJ and Mawe GM: Innervation of the extrahepatic biliary tract. Anat Rec A Discov Mol Cell Evol Biol 280: 836-847, 2004.

119. Keast JR, Furness JB and Costa M: Distribution of certain peptide-containing nerve fibres and endocrine cells in the gastrointestinal mucosa in five mammalian species. J Comp Neurol 236: 403-422, 1985.

120. Cai WQ and Gabella G: Innervation of the gall bladder and biliary pathways in the guinea-pig. J Anat 136: 97-109, 1983.

121. Talmage EK, Pouliot WA, Schemann M and Mawe GM: Structure and chemical coding of human, canine and opossum gallbladder ganglia. Cell Tissue Res 284: 289-302, 1996.

122. Gilloteaux J, Pomerants B and Kelly TR: Human gallbladder mucosa ultrastructure: Evidence of intraepithelial nerve structures. Am J Anat 184: 321-333, 1989.

123. De Giorgio R, Zittel TT, Parodi JE, Becker JM, Brunicardi FC, Go VL, Brecha NC and Sternini C: Peptide immunoreactivities in the ganglionated plexuses and nerve fibers innervating the human gallbladder. J Auton Nerv Syst 51: 37-47, 1995.

124. De Giorgio R, Parodi JE, Brecha NC, Brunicardi FC, Becker JM, Go VL and Sternini C: Nitric oxide producing neurons in the monkey and human digestive system. J Comp Neurol 342: 619-627, 1994.

125. Mawe GM and Ellis LM: Chemical coding of intrinsic and extrinsic nerves in the guinea pig gallbladder: Distributions of PACAP and orphanin FQ. Anat Rec 262: 101-109, 2001.

126. Talmage EK, Pouliot WA, Cornbrooks EB and Mawe GM: Transmitter diversity in ganglion cells of the guinea pig gallbladder: An immunohistochemical study. J Comp Neurol 317: 45-56, 1992.

127. Sundler F, Alumets J, Håkanson R, Ingemansson S, Fahrenkrug J and Schaffalitzky de Muckadell O: VIP innervation of the gallbladder. Gastroenterology 72: 1375-1377, 1977.

128. Uemura S, Pompolo S, Furness JB and Hardy KJ: Nitric oxide synthase in neurons of the human gall-bladder and its colocalization with neuropeptides. J Gastroenterol Hepatol 12: 257-265, 1997.

129. Hopton DS: The influence of the vagus nerves on the biliary system. Br J Surg 60: 216-218, 1973.

130. Mawe GM, Moses PL, Saccone GTP and Pozo MJ: Motility of the Biliary Tract. In: Textbook of Gastroenterology, pp264-283, 2008.

131. Schjoldager B, Molero X and Miller LJ: Functional and biochemical characterization of the human gallbladder muscularis cholecystokinin receptor. Gastroenterology 96: 1119-1125, 1989.

132. Xu D, Yu BP, Luo HS and Chen LD: Control of gallbladder contractions by cholecystokinin through cholecystokinin-a receptors on gallbladder interstitial cells of Cajal. World J Gastroenterol 14: 2882-2887, 2008.

133. Mawe GM, Gokin AP and Wells DG: Actions of cholecystokinin and norepinephrine on vagal inputs to ganglion cells in guinea pig gallbladder. Am J Physiol 267: G1146-G1151, 1994.

134. Behar J and Biancani P: Pharmacologic characterization of excitatory and inhibitory cholecystokinin receptors of the cat gallbladder and sphincter of Oddi. Gastroenterology 92: 764-770, 1987.

135. Stengel PW and Cohen ML: Muscarinic receptor knockout mice: Role of muscarinic acetylcholine receptors M(2), M(3), and M(4) in carbamylcholine-induced gallbladder contractility. J Pharmacol Exp Ther 301: 643-650, 2002.

136. Takahashi T, Kurosawa S and Owyang C: Regulation of PI hydrolysis and cAMP formation by muscarinic M3 receptor in guinea pig gallbladder. Am J Physiol 267: G523-G528, 1994.

137. Lee MC, Yang YC, Chen YC and Huang SC: Muscarinic receptor M3 mediates human gallbladder contraction through voltage-gated Ca^{2+} channels and Rho kinase. Scand J Gastroenterol 48: 205-212, 2013.

138. Patacchini R and Maggi CA: Effect of newly developed tachykinin agonist and antagonists on the guinea pig isolated gallbladder. J Pharmacol Exp Ther 261: 191-194, 1992.

139. Yau WM: Mode of stimulation of gallbladder contraction by substance K. Am J Physiol 259: G838-G841, 1990.

140. O'Riordan AM, Quinn T and Baird AW: Role of prostaglandin E(2) and Ca(2+) in bradykinin induced contractions of guinea-pig gallbladder in vitro. Eur J Pharmacol 431: 245-252, 2001.

141. Trevisani M, Amadesi S, Schmidlin F, Poblete MT, Bardella E, Maggiore B, Harrison S, Figueroa CD, Tognetto M, Navarra G, et al: Bradykinin B2 receptors mediate contraction in the normal and inflamed human gallbladder in vitro. Gastroenterology 125: 126-135, 2003.

142. Andre E, Gazzieri D, Bardella E, Ferreira J, Mori MA, Saul VV, Bader M, Calixto JB, De Giorgio R, Corinaldesi R, *et al*: Expression and functional pharmacology of the bradykinin B1 receptor in the normal and inflamed human gallbladder. Gut 57: 628-633, 2008.

143. Takahashi T, Kusunoki M, Ishikawa Y, Kantoh M, Yamamura T and Utsunomiya J: Adenosine 5'-triphosphate release evoked by electrical nerve stimulation from the guinea-pig gallbladder. Eur J Pharmacol 134: 77-82, 1987.

144. Puchałowicz K, Tarnowski M, Baranowska-Bosiacka I, Chlubek D and Dziedziejko V: P2X and P2Y receptors-role in the pathophysiology of the nervous system. Int J Mol Sci 15: 23672-23704, 2014.

145. Bartoo AC, Nelson MT and Mawe GM: ATP induces guinea pig gallbladder smooth muscle excitability via the P2Y4 receptor and COX-1 activity. Am J Physiol Gastrointest Liver Physiol 294: G1362-G1368, 2008.

146. Ruan HZ and Burnstock G: P2X2 and P2X3 receptor expression in the gallbladder of the guinea pig. Auton Neurosci 111: 89-96, 2004.

147. Rasmussen TN, Harling H, Rehfeld JF and Holst JJ: Calcitonin gene-related peptide (CGRP), a potent regulator of biliary flow. Neurogastroenterol Motil 9: 215-220, 1997.

148. Kline LW and Pang PK: Cyclic AMP modulates part of the relaxant action of calcitonin gene-related peptide in guinea pig gallbladder strips. Regul Pept 72: 55-59, 1997.

149. Kline LW and Pang PK: Nitric oxide modulates the calcitonin gene-related peptide-induced relaxation in guinea pig gall-bladder strips in vitro. Regul Pept 50: 207-212, 1994.

150. Gultekin H, Erdem SR, Emre-Aydingoz S and Tuncer M: The role of nitric oxide in the electrical field stimulation-induced contractions of sphincter of oddi and gallbladder strips in Guinea pigs. J Pharmacol Sci 101: 240-244, 2006.

151. Alcón S, Morales S, Camello PJ, Salido GM, Miller SM and Pozo MJ: Relaxation of canine gallbladder to nerve stimulation involves adrenergic and non-adrenergic non-cholinergic mechanisms. Neurogastroenterol Motil 13: 555-566, 2001.

152. Xue L, Farrugia G, Miller SM, Ferris CD, Snyder SH and Szurszewski JH: Carbon monoxide and nitric oxide as coneurotransmitters in the enteric nervous system: Evidence from genomic deletion of biosynthetic enzymes. Proc Natl Acad Sci USA 97: 1851-1855, 2000.

153. Harmar AJ, Fahrenkrug J, Gozes I, Laburthe M, May V, Pisegna JR, Vaudry D, Vaudry H, Waschek JA and Said SI: Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR review 1. Br J Pharmacol 166: 4-17, 2012.

154. Pálvölgyi A, Sári R, Németh J, Szabolcs A, Nagy I, Hegyi P, Lonovics J and Szilvássy Z: Interplay between nitric oxide and VIP in CCK-8-induced phasic contractile activity in the rabbit sphincter of Oddi. World J Gastroenterol 11: 3264-3266, 2005.

155. Pang PK and Kline LW: Protein kinase C mediates the contractile actions of pituitary adenylate cyclase activating polypeptide in guinea pig gallbladder strips. Regul Pept 77: 63-67, 1998.

156. Greaves RR, O'Donnell LJ, Battistini B, Forget MA and Farthing MJ: The differential effect of VIP and PACAP on guinea pig gallbladder in vitro. Eur J Gastroenterol Hepatol 12: 1181-1184, 2000.

157. Parkman HP, Pagano AP and Ryan JP: Dual effects of PACAP on guinea pig gallbladder muscle via PACAP-preferring and VIP/PACAP-preferring receptors. Am J Physiol 272: G1433-G1438, 1997.

158. Fisher RS, Rock E, Levin G and Malmud L: Effects of somatostatin on gallbladder emptying. Gastroenterology 92: 885-890, 1987.

159. Greenberg GR, McCloy RF, Adrian TE, Chadwick VS, Baron JH and Bloom SR: Inhibition of pancreas and gallbladder by pancreatic polypeptide. Lancet 2: 1280-1282, 1978.

160. Walker JP, Khalil T, Wiener I, Fagan CJ, Townsend CM Jr, Greeley GH Jr and Thompson JC: The role of neurotensin in human gallbladder motility. Ann Surg 201: 678-683, 1985.

161. Holzer P, Reichmann F and Farzi A: Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut-brain axis. Neuropeptides 46: 261-274, 2012.

162. Chen Q, Lee K, Xiao Z, Biancani P and Behar J: Mechanism of gallbladder relaxation in the cat: Role of norepinephrine. J Pharmacol Exp Ther 285: 475-479, 1998.

163. McGowan BMC and Bloom SR: Peptide YY and appetite control. Curr Opin Pharmacol 4: 583-588, 2004.

164. Hoentjen F, Hopman WP and Jansen JB: Effect of circulating peptide YY on gallbladder emptying in humans. Scand J Gastroenterol 36: 1086-1091, 2001.

165. Hazelwood RL: The pancreatic polypeptide (PP-fold) family: Gastrointestinal, vascular, and feeding behavioral implications. Proc Soc Exp Biol Med 202: 44-63, 1993.

166. Kojima S, Ueno N, Asakawa A, Sagiyama K, Naruo T, Mizuno S and Inui A: A role for pancreatic polypeptide in feeding and body weight regulation. Peptides 28: 459-463, 2007.

167. Vu MK, Van Oostayen JA, Biemond I and Masclee AA: Effect of somatostatin on postprandial gallbladder relaxation. Clin Physiol 21: 25-31, 2001.

168. Maselli MA, Piepoli AL, Pezzolla F, Caruso ML and Lorusso D: Effect of somatostatin on human gallbladder motility: An in vitro study. Neurogastroenterol Motil 11: 47-53, 1999.

169. Milenov K, Vassileva M, Marinova D and Kalfin R: Effect of neurotensin on the canine gallbladder motility: In vivo and in vitro experiments. Neuropeptides 25: 233-239, 1993.

170. Yamasato T and Nakayama S: Effects of neurotensin on the motility of the isolated gallbladder, bile duct and ampulla in guinea-pigs. Eur J Pharmacol 148: 101-106, 1988.

171. Feeley TM, Clanachan AS and Scott GW: Contractility of human gallbladder muscle in vitro. Aliment Pharmacol Ther 1: 607-616, 1987.

172. Lavoie B, Balemba OB, Godfrey C, Watson CA, Vassileva G, Corvera CU, Nelson MT and Mawe GM: Hydrophobic bile salts inhibit gallbladder smooth muscle function via stimulation of GPBAR1 receptors and activation of KATP channels. J Physiol 588: 3295-3305, 2010.

173. Jain AK, Stoll B, Burrin DG, Holst JJ and Moore DD: Enteral bile acid treatment improves parenteral nutrition-related liver disease and intestinal mucosal atrophy in neonatal pigs. Am J Physiol Gastrointest Liver Physiol 302: G218-G224, 2012.

174. Yusta B, Matthews D, Flock GB, Ussher JR, Lavoie B, Mawe GM and Drucker DJ: Glucagon-like peptide-2 promotes gallbladder refilling via a TGR5-independent, GLP-2R-dependent pathway. Mol Metab 6: 503-511, 2017.

175. Kliewer SA and Mangelsdorf DJ: Bile acids as hormones: The FXR-FGF15/19 pathway. Dig Dis 33: 327-331, 2015.

176. Everhart JE and Ruhl CE: Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. Gastroenterology 136: 1134-1144, 2009.

177. Cai JS, Qiang S and Bao-Bing Y: Advances of recurrent risk factors and management of choledocholithiasis. Scand J Gastroenterol 52: 34-43, 2017.

178. Behar J, Lee KY, Thompson WR and Biancani P: Gallbladder contraction in patients with pigment and cholesterol stones. Gastroenterology 97: 1479-1484, 1989.

179. Carey MC and Small DM: The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. J Clin Invest 61: 998-1026, 1978.

180. Zheng Y, Xu M, Heianza Y, Ma W, Wang T, Sun D, Albert CM, Hu FB, Rexrode KM, Manson JE and Qi L: Gallstone disease and increased risk of mortality: Two large prospective studies in US men and women. J Gastroenterol Hepatol 33: 1925-1931, 2018.

181. Huang L, Ding C and Si X: Changes in the interstitial cells of Cajal in the gallbladder of guinea pigs fed a lithogenic diet. Exp Ther Med 22: 823, 2021.

182. Xiao ZL, Chen Q, Amaral J, Biancani P and Behar J: Defect of receptor-G protein coupling in human gallbladder with cholesterol stones. Am J Physiol Gastrointest Liver Physiol 278: G251-G258, 2000.

183. Gao G, Ding ZQ and Zou SQ: The changes of vasoactive intestinal polypeptide and VIPR expression in the patients with cholesterol gallstone. J Clinc Surg 12: 224-226, 2004.

184. Wang HH, Portincasa P and Wang DQH: Update on the molecular mechanisms underlying the effect of cholecystokinin and cholecystokinin-1 receptor on the formation of cholesterol gallstones. Curr Med Chem 26: 3407-3423, 2019.

185. Tan YY: Studies on the role of Cajal interstitial cell in cholecystolithiasis and surgical methodology of endoscopic minimal invasive cholecystolithotomy. Dongnan Daxue: 58-91, 2015.

186. Fu BB, Xu JH, Wu SD and Fan Y: Effect of cholesterol on in vitro cultured interstitial Cajal-like cells isolated from guinea pig gallbladders. World J Gastrointest Surg 12: 226-235, 2020.

187. Lavoie B, Nausch B, Zane EA, Leonard MR, Balemba OB, Bartoo AC, Wilcox R, Nelson MT, Carey MC and Mawe GM: Disruption of gallbladder smooth muscle function is an early feature in the development of cholesterol gallstone disease. Neurogastroenterol Motil 24: e313-e324, 2012.

188. Chen Q, Amaral J, Biancani P and Behar J: Excess membrane cholesterol alters human gallbladder muscle contractility and membrane fluidity. Gastroenterology 116: 678-685, 1999.

189. Wang B, Ding YM, Wang CT and Wang WX: Role of ROCK expression in gallbladder smooth muscle contraction. Mol Med Rep 12: 2907-2911, 2015.

190. Xu QW, Freedman SM and Shaffer EA: Inhibitory effect of bile salts on gallbladder smooth muscle contractility in the guinea pig in vitro. Gastroenterology 112: 1699-1706, 1997.

191. Rutishauser SC: Effects of bile salts on the motor activity of the guinea-pig gall-bladder in vitro. Q J Exp Physiol Cogn Med Sci 63: 265-276, 1978.

192. Pasternak A, Matyja A, Gil K, Gajda M, Tomaszewski KA, Gajda M, Tomaszewski KA, Matyja M, Walocha JA and Kulig J: Interstitial cajal-like cells and bile lithogenicity in the pathogenesis of gall-stone disease. Pol Przegl Chir 85: 311-316, 2013.

193. Villanacci V, Del Sordo R, Salemme M, Cadei M, Sidoni A and Bassotti G: The enteric nervous system in patients with calculous and acalculous gallbladder. Dig Liver Dis 48: 792-795, 2016.

194. Fu Y, Pang L, Dai W, Wu S and Kong J: Advances in the study of acute acalculous cholecystitis: A comprehensive review. Dig Dis 40: 468-478, 2022.

195. Cong P, Xiao ZL, Biancani P and Behar J: Prostaglandins mediate tonic contraction of the guinea pig and human gallbladder. Am J Physiol Gastrointest Liver Physiol 292: G409-G418, 2007.

196. Xiao ZL, Amaral J, Biancani P and Behar J: Impaired cytoprotective function of muscle in human gallbladders with cholesterol stones. Am J Physiol Gastrointest Liver Physiol 288: G525-G532, 2005.

197. Myers SI, Bartula LL, Colvin MP, Parkman HP, Braverman AA and Ruggieri MR: Bile duct ligation induced acute inflammation up-regulates cyclooxygenase-2 content and PGE2 release in guinea pig gallbladder smooth muscle cell cultures. Prostaglandins Leukot Essent Fatty Acids 72: 327-333, 2005.

198. Parkman HP, James AN, Thomas RM, Bartula LL, Ryan JP and Myers SI: Effect of indomethacin on gallbladder inflammation and contractility during acute cholecystitis. J Surg Res 96: 135-142, 2001.

199. Jennings LJ and Mawe GM: PGE2 hyperpolarizes gallbladder neurons and inhibits synaptic potentials in gallbladder ganglia. Am J Physiol 274: G493-G502, 1998.

200. Parkman HP, James AN, Bogar LJ, Bartula LL, Thomas RM, Ryan JP and Myers SI: Effect of acalculous cholecystitis on gallbladder neuromuscular transmission and contractility. J Surg Res 88: 186-192, 2000.

201. Xiao ZL, Andrada MJ, Biancani P and Behar J: Reactive oxygen species (H(2)O(2)): Effects on the gallbladder muscle of guinea pigs. Am J Physiol Gastrointest Liver Physiol 282: G300-G306, 2002.

202. Cullen JJ, Conklin JL, Ephgrave KS and Oberley LW: The role of antioxidant enzymes in the control of opossum gallbladder motility. J Surg Res 86: 155-161, 1999.

203. Pozo MJ, Camello PJ and Mawe GM: Chemical mediators of gallbladder dysmotility. Curr Med Chem 11: 1801-1812, 2004.

204. Jennings LJ, Salido GM, Pozo MJ, Davison JS, Sharkey KA, Lea RW and Singh J: The source and action of histamine in the isolated guinea-pig gallbladder. Inflamm Res 44: 447-453, 1995.

205. Al-Jiffry BO, Shaffer EA, Woods CM, Menadue M, Young F, Oliver J, Thomas AC, Toouli J and Saccone GT: Endogenous endothelin increases gallbladder tone and leads to acute cholecystitis in the Australian possum. Neurogastroenterol Motil 16: 125-133, 2004.

206. Huang SC, Lee MC, Wei CK and Huang SM: Endothelin receptors in human and guinea-pig gallbladder muscle. Regul Pept 98: 145-153, 2001.

207. Huang ZP, Qiu H, Yang Y and Yu BP: Effect of neutrophils on gallbladder interstitial cajal-like cells in guinea pig model of acute cholecystitis. Cell Physiol Biochem 39: 2033-2043, 2016.

208. Lin MJ, Chen L, Huang ZP, Qiu H and Yu BP: Neutrophils injure gallbladder interstitial Cajal-like cells in a guinea pig model of acute cholecystitis. J Cell Physiol 234: 4291-4301, 2019.

209. Burns AJ, Herbert TM, Ward SM and Sanders KM: Interstitial cells of Cajal in the guinea-pig gastrointestinal tract as revealed by c-Kit immunohistochemistry. Cell Tissue Res 290: 11-20, 1997.

210. Christensen J: A commentary on the morphological identification of interstitial cells of Cajal in the gut. J Auton Nerv Syst 37: 75-88, 1992.

211. Ward SM, Burke EP and Sanders KM: Use of rhodamine 123 to label and lesion interstitial cells of Cajal in canine colonic circular muscle. Anat Embryol (Berl) 182: 215-224, 1990.

212. Mikkelsen HB, Thuneberg L and Wittrup IH: Selective double staining of interstitial cells of Cajal and macrophage-like cells in small intestine by an improved supravital methylene blue technique combined with FITC-dextran uptake. Anat Embryol (Berl) 178: 191-195, 1988.

213. Xue C, Ward SM, Shuttleworth CW and Sanders KM: Identification of interstitial cells in canine proximal colon using NADH diaphorase histochemistry. Histochemistry 99: 373-384, 1993.

214. Huang Y, Mei F, Yu B, Zhang HJ, Han J, Jiang ZY and Zhou DS: Distribution of the interstitial Cajal-like cells in the gallbladder and extrahepatic biliary duct of the guinea-pig. Acta Histochem 111: 157-165, 2009.

215. Vannucchi MG and Traini C: Interstitial cells of Cajal and telocytes in the gut: Twins, related or simply neighbor cells? Biomol Concepts 7: 93-102, 2016.

216. Sugai M: Morphological studies of human gallbladder. Nihon Heikatsukin Gakkai Zasshi 21: 119-138, 1985 (In Japanese).

217. Hartshorne DJ, Ito M and Erdödi F: Myosin light chain phosphatase: Subunit composition, interactions and regulation. J Muscle Res Cell Motil 19: 325-341, 1998.

218. Mnh: Histologie du système nerveux de l'homme et des vertébrés. J Neuropathol Exp Neurol 57: 883, 1998.

219. Cawston EE and Miller LJ: Therapeutic potential for novel drugs targeting the type 1 cholecystokinin receptor. Br J Pharmacol 159: 1009-1021, 2010.

220. Zhang L, Bonev AD, Mawe GM and Nelson MT: Protein kinase A mediates activation of ATP-sensitive K+ currents by CGRP in gallbladder smooth muscle. Am J Physiol 267: G494-G499, 1994.

221. Luman W, Ardill JE, Armstrong E, Smith GD, Brett L, Lessells AM, Haynes WG, Gray GA, Mickley EJ, Webb DJ and Palmer KR: Nitric oxide and gall-bladder motor function. Aliment Pharmacol Ther 12: 425-432, 1998.

222. Farrugia G, Miller SM, Rich A, Liu X, Maines MD, Rae JL and Szurszewski JH: Distribution of heme oxygenase and effects of exogenous carbon monoxide in canine jejunum. Am J Physiol 274: G350-G358, 1998.

223. Zhang ZH, Qin CK, Wu SD, Xu J, Cui XP, Wang ZY and Xian GZ: Roles of sphincter of Oddi motility and serum vasoactive intestinal peptide, gastrin and cholecystokinin octapeptide. World J Gastroenterol 20: 4730-4736, 2014.

224. Morales S, Camello PJ, Mawe GM and Pozo MJ: Cyclic AMP-mediated inhibition of gallbladder contractility: Role of K+ channel activation and Ca^{2+} signaling. Br J Pharmacol 143: 994-1005, 2004.

225. Bitar KN and Makhlouf GM: Relaxation of isolated gastric smooth muscle cells by vasoactive intestinal peptide. Science 216: 531-533, 1982.

226. Choi M, Moschetta A, Bookout AL, Peng L, Umetani M, Holmstrom SR, Suino-Powell K, Xu HE, Richardson JA, Gerard RD, et al: Identification of a hormonal basis for gallbladder filling. Nat Med 12: 1253-1255, 2006.

227. Yamasaki T, Chijiiwa K and Chijiiwa Y: Somatostatin inhibits cholecystokinin-induced contraction of isolated gallbladder smooth muscle cells. J Surg Res 59: 743-746, 1995.

228. Kaczmarek P, Singh V, Cashen DE, Yang L, Berk S, Pasternak A, Xiong Y, Shen DM, Hutchins SM, Chapman K, et al: Somatostatin receptor subtypes 2 and 5 mediate inhibition of egg yolk-induced gall bladder emptying in mice. Neurogastroenterol Motil 22: 204-209, e66, 2010.