Abstract. Calcium participates in most of the biological processes in the human body. The calcium sensing receptor (CaSR), as an important regulator of calcium homeostasis, is expressed in all of the organs of the digestive system. CaSR plays a key role in gastrointestinal physiological function and in the occurrence of digestive disease. For example, the inactivation or mutation of the CaSR gene usually leads to one of several disorders of calcium metabolism. High dietary Ca²⁺ may stimulate CaSR activation and could both inhibit tumor development and increase the chemotherapeutic sensitivity of cancer cells in colon cancer tissues. Further, CaSR has also been reported to have a potential role in the treatment for diarrheal diseases and the form of pancreatitis that is associated with carbonate stones. Therefore, CaSR is an important target for treating digestive diseases, and the calcimimetics (CaSR agonist) have been confirmed as practical, feasible and effective clinical therapies for hyperparathyroidism. This review intends to explore the role of CaSR in digestive physiology and pathophysiology as well as current treatments utilizing CaSR-based therapeutics.

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1. Introduction

Ca²⁺ is a ubiquitous cellular signal. Changes in intracellular Ca²⁺ control various cellular processes that are relevant to regulating normal function and to developing diseases. Examples of such processes include blood coagulation, nervous excitation, angiogenesis, cell apoptosis and the development of cancer (1). Ca²⁺ also plays an important role in the physiological function and pathological processes of the digestive system. The calcium-sensing receptor (CaSR) is a member of the G protein-coupled receptor (GPCR) C superfamily and plays a key role in maintaining systemic calcium homeostasis (2). It is expressed in diverse mammalian tissues, including the parathyroid gland, the cardiovascular system and the entire digestive system (3), and it mediates insulin and gastric acid secretions as well as intestinal fluid transport (4). A mutation in the CaSR gene might be a predisposing factor that leads to an increased susceptibility of chronic and recurrent acute pancreatitis (5). Further, the CaSR gene is believed to be an antitumor factor and is decreased or even absent in adults with colorectal cancer (34). However, the mechanism and function of CaSR in the gastrointestinal tract have not yet been completely elucidated, particularly relating to certain digestive diseases and tumors. To support CaSR-based therapeutics of digestive diseases, we need to deepen our understanding of CaSR structure and its biochemical features and to determine the role of CaSR in the physiological and pathophysiological processes of the gastrointestinal system.

2. The structural features of CaSR

CaSR, a member of the C family of G protein-coupled receptors (GPCR), was first cloned from the bovine parathyroid gland (6). The human CaSR gene is located on the long arm of chromosome 3, and it encodes a polypeptide composed of 1,078 amino acids. CaSR can be divided into three parts: the extracellular
domain (ECD), the transmembrane domain (TMD) and the intracellular domain. The extracellular domain (ECD) (N terminal) includes 612 amino acids, which contains the binding site of Ca²⁺ or a CaSR agonist. The transition from the activation to the deactivation of CaSR mostly occurs in the ECD. Similar to other GPCRs, CaSR has a 250-amino-acid transmembrane domain (TMD) that has 7 transmembrane helices (7,8). A cysteine-rich domain exists between the ECD and the first transmembrane domain of TMD, removing this domain results in the loss of CaSR signaling (9). The final 216 amino acids form the intracellular tail (C terminal) that contains protein kinase C, A and D phosphorylation sites (7). CaSR agonists include polyvalent metal cations (such as Ca²⁺ and Gd³⁺) and organic polycations such as polylysine, protamine and L-amino acids.

Because the concentration of Ca²⁺ in the blood does not change obviously, the complex structure and many binding sites of CaSR are required to monitor and regulate the blood Ca²⁺ concentration. Hu and Spiegel (8) and Huang et al (10) reported that the molecular structure of the ECD of CaSR is bilobed, where every two ECDs form a Venus flytrap (VFT) that has a binding site for Ca²⁺ in the crevice of the two lobes. The TMD also contains the Ca²⁺ binding site to regulate when the ECD is inactivated (11). Several transcription factors take part in modulating the activation of CaSR, such as vitamin D response elements (VDRE), Stat1/3, NFκB and Sp1/3, and their binding sites are located in the CaSR promoters (12,13).

3. The distribution of CaSR and its function in Ca²⁺ homeostasis and cellular processes

To date, studies have confirmed the important role of CaSR in Ca²⁺ homeostasis (2,14), indicating that CaSR is expressed in most organs that regulate the Ca²⁺ balance, including the parathyroid glands, kidney, thyroid C cells, stomach, osteoblasts and osteoclasts (15-17). CaSR was also shown to be widely expressed in the nervous system, esophagus, liver, pancreas, pituitary gland, peripheral blood, breast and arterial smooth muscle cells (15,18-20). It is well known that several factors affect the mechanism of Ca²⁺ homeostasis, including Ca²⁺ sensors in the cell membrane and Ca²⁺ regulating hormones. CaSR is an important Ca²⁺ sensor that not only regulates the release of Ca²⁺ from the endoplasmic reticulum (ER) but also controls the switch of the Ca²⁺ channel in the cellular membrane, which allows the transport of Ca²⁺ into or out of the extracellular fluid (21,22). For example, CaSR activation can mediate Ca²⁺ entry into human aortic smooth muscle cells via transient receptor potential 6 (TRPC6) (21). TRPC3, another member of the TRPC family, was proved to participate in the CaSR mediation of Ca²⁺ overload in the heart (23). In addition, hormones play an important role in regulating Ca²⁺ balance. CaSR has been proven to regulate the secretion and production of hormones, including parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxyvitamin D 3 [1,25(OH)23], that maintain Ca²⁺ homeostasis (24,25). Taken together, in physiological conditions, CaSR is critical to the Ca²⁺ balance in the human body.

The CaSR acts through at least two G proteins (Gαq and Gαq/11) to regulate multiple intracellular second messengers, including inositol trisphosphate (IP₃), cytoplasmic free Ca²⁺ ([Ca²⁺]cyt) and cyclic adenosine monophosphate (cAMP) (26). The activated CaSR can stimulate the phospholipase C (PLC-IP3) signal pathway to prompt the release of Ca²⁺ from endoplasmic reticulum (ER) (27). The intracellular signal pathways including mitogen activated protein kinases (MAPKs), phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT), and phospholipases A, C and D can also be activated by the activation of CaSR (27,28), then regulate several cellular processes that are involved in cellular secretion, proliferation, differentiation, chemotaxis, apoptosis, gene expression, ion channel switch and aging (3,29,30). Numerous transcription factors participate in the complex regulation process that depends on CaSR. For example, activated CaSR can regulate the cyclin D family genes, c-Myc and c-Fos, to affect cell proliferation (31,32), and NFκB can also be stimulated by CaSR to promote the secretion of proinflammatory factors and chemokines that cause the development of obesity induced adipose tissue dysfunction disease (33). CaSR was reported to decrease β-catenin-TCF-4 complex formation and thus suppress the development of tumors in colon cancer (34). In summary, the CaSR is considered to be a critical player in cellular processes.

4. Role of CaSR in the esophagus

CaSR is known to be expressed in the basal cells of the human esophagus and in the non-tumorigenic esophageal epithelial cell line HET-1A (35,36). In the study of the HET-1A cell line, activated CaSR increases intracellular Ca²⁺ concentration mobilization and activates ERK1/2 (MAPK) signal pathways to promote the multifunctional cytokine IL-8 (CX-CL8) secretion (36). In oesophagitis, Mulder et al proved that CaSR can be activated by eosinophil-released major basic protein (MBP) to promote fibroblast growth factor 9 (FGF9) secretion and that it then affects the downstream proliferation-related genes BMP-2 and BMP-4 to induce the proliferation of esophageal epithelial cells (37). CaSR may be implicated in the proliferative response to injury and the pathogenesis of oesophagitis. However, it is not yet known whether the CaSR activation affects the proliferation or differentiation of cancer cells in animals or human esophageal cancer.

5. CaSR in the liver

Much evidence shows that CaSR is important for normal hepatic physiological function and liver diseases. In 2001, Canaff et al first identified expression in liver tissue and primary cultured hepatocytes (20). Subsequently research demonstrated the functional expression of CaSR in Buffalo rat liver cells and that activated CaSR stimulated bile flow by the PLC-IP3 signal transduction pathway (38). In the hepatic ischemia/reperfusion (I/R) injury model, CaSR activation induced cell apoptosis by promoting the p38 MAPK and ERK-1/2 signal pathway phosphorylation and regulating the downstream Bel-2, Cyt-c, caspase-3 and Bax gene expression (39). Much more importantly, in the cirrhotic animal model which was induced by carbon tetrachloride (CCL4), CaSR has been reported to reduce the intrahepatic resistance to portal flow (40). Thus, the regulation of CaSR might serve as a new pharmacological target for the prevention and treatment of drug- or alcohol-induced liver disease.
6. CaSR and the pancreas

In 2002, Rácz et al (19) were the first to report evidence for the presence of CaSR in the normal human pancreas, pancreatic cancer and chronic pancreatitis tissue. Immunohistochemistry located the expression of CaSR in the endocrine and exocrine pancreas, including β-islet cells, duct cell, acinar cells and various non-exocrine cells such as intrapancreatic nerves and blood vessels (19,41). These data suggest that CaSR has a function in the human pancreas. However, the CaSR function varies depending on where it is located in the pancreas (Fig. 1).

CaSR in exocrine and endocrine pancreas physiology. The function of the exocrine pancreas mainly include the secretion of various digestive enzymes and pancreatic juice. CaSR was observed to be highly expressed in human pancreatic acinar and ducts cells (19), suggesting a potential role for CaSR in regulating pancreatic exocrine function. In 1999, Bruce et al reported that the activation of CaSR in the rat pancreas duct luminal membrane increased ductal HCO₃⁻ secretion (41). Moreover, CaSR could monitor and regulate the Ca²⁺ concentration in pancreatic juice by triggering ductal electrolyte and fluid secretion, thereby reducing the precipitation of Ca²⁺ salts in the duct lumen and decreasing both the risk of carbonate stone formation and the progression to pancreatitis (19). Although the expression of CaSR could be detected in human acinar cells (42), the function of the receptor in this setting is not yet clear.

The function of CaSR in pancreatic endocrine tissue is confirmed to participate in the regulation of glucose-induced insulin secretion by β-cells (42-44). It is known that change in plasma glucose concentration is the major factor stimulating insulin release. The metabolism of glucose could change the ATP/ADP ratio by closing ATP-dependent K⁺ channels that then open the voltage-gated Ca²⁺ channels (VGCC) to mediate intracellular Ca²⁺ changes and induce insulin release (45). The extracellular Ca²⁺ levels were previously observed to induce transitory increases in insulin secretion (46). The experiments of Squires et al (45) and Gray et al (47) in human islets and insulin-secreting cell lines (MIN6) also confirmed that low concentrations of Ca²⁺ could induce a marked but transient insulin secretion by activating the CaSR and the downstream MAPK signal pathway. In addition, CaSR was shown to affect the β-cell proliferation and cell-cell communication that cooperate in insulin secretory responses (48). Hills et al showed that CaSR was able to enhance the function of the epithelial adhesion protein (E-cadherin) in β-cells and the expression of L-type VDCC, which reinforces cell-cell adhesion and β-cell function to promote the insulin secretion of neighboring cells. However, the role of CaSR is dual because they have a functional that varies between enhancing and inhibiting insulin release. Studies have shown that CaSR in human β-cells have a negative modulatory effect on insulin secretion (43). First, the CaSR is activated by extracellular Ca²⁺ which is a promotive factor for insulin secretion, but if there is a prolonged Ca²⁺ concentration-dependent activation, this is reversed and inhibition of insulin secretion is affected. Moreover, the transduction mechanism is confirmed to be unlikely through the cAMP or PLC-IP₃ pathways. Squires et al (43) proposed that coupled receptor is the possible mechanism for insulin secretion inhibition, but they have not yet garnered enough evidence to prove this hypothesis. Although it has been reported that the colocalization and the spatial interactions between the L-type voltage-dependent calcium channels (L-type VDCC) and CaSR enhanced the glucose-induced the secretion of insulin (45), the latest report found that the CaSR that is activated by L-histidine depresses L-type VDCC activity and inhibits insulin secretion in β-cells (49). Thus, the different stimulation factors and space conformational changes of CaSR may be a possible explanation for the role of CaSR in negatively modulating insulin secretion.

The role of CaSR in pancreatic disease. Recent studies have primarily focused on the acute and chronic pancreatitis (CP)
associated with CaSR receptor mutation (6,50,51). The mutations in the CaSR gene and the pancreatic secretory trypsin inhibitor gene (SPINK1) were identified in patients with familial hypocalciuric hypercalciemia (FHH), in 2010, genetic linkage and candidate gene studies recognized that these two gene mutations were associated with susceptibility to acute and/or chronic pancreatitis (51,52). Felderbauer et al found that mutations of the CaSR gene (R896H) were particularly associated with SPINK1-related chronic pancreatitis (51). Clinical research has suggested that pancreatitis patients usually have both the CaSR mutation and the N34S SPINK1 gene mutation, whereas in healthy patients, only an isolated CaSR or N34S SPINK1 gene mutation can be detected. This finding suggests that the CaSR gene is an important co-factor in SPINK1-related pancreatitis. In 2008, Murugaian et al found that four new mutations of the CaSR gene in patients in India with tropical chronic pancreatitis, which confirmed that CaSR variants could lead to idiopathic CP with or without SPINK1 mutations (53). Moreover, some literature has elucidated the role of CaSR in pancreatic cancer. CaSR was detected in human pancreatic cancer tissue and cell lines. Early studies reported that CaSR is activated by elevated extracellular Ca\textsuperscript{2+} or Gd\textsuperscript{4+} that can significantly reduce cell proliferation in the well-differentiated human pancreatic adenocarcinoma capan-1 cell line (19), but these results were obtained in vitro. More clinical evidence is needed to determine the role of CaSR in pancreatic tumors.

7. CaSR expression in the stomach

The regulatory function of Ca\textsuperscript{2+} and CaSR in gastric acid secretion. The stomach is an important digestive organ. Gastric acid plays an indispensable role in modulating digestion and absorption, and an imbalance usually induces gastrointestinal disease (54). Generally, the classically modulated pathways of acid secretion are involved including the paracrine, endocrine, and neuroendocrine systems (55), in addition, the H\textsuperscript{+}-K\textsuperscript{-}-ATPase activation was also shown to be crucial to the formation of HCL and the regulation of gastric acid secretion (56,57). Ca\textsuperscript{2+} is an important second messenger and has a role in the above process. In previous animal and human studies, experiments have proved that Ca\textsuperscript{2+} and amino acids are useful in stimulating acid secretion. High levels of intravenous or intestinal Ca\textsuperscript{2+} both promote the release of gastric acid (57,58). Interestingly, we found that gastric acid increases that are induced by hormonal or neuronal stimulation accompany an elevation in intracellular Ca\textsuperscript{2+} levels that can be detected in the gastric gland (59,60). Thus, there is a close connection between Ca\textsuperscript{2+} and gastric acid secretion, and the CaSR is confirmed to participate in the regulation of Ca\textsuperscript{2+}-induced gastric acid secretion.

The role of CaSR in regulating gastric acid secretion has been proven in both in vivo and in vitro experiments, and gastric cells of the basement membrane, mucus cells, G cells and D cells all express CaSR (61). Ceglia et al provided the first in vivo evidence that the activation of the CaSR increases the serum gastrin levels and basal gastric acid secretion in healthy older men and women (62). Activated CaSR increased the intracellular Ca\textsuperscript{2+} concentration by leading to a release of Ca\textsuperscript{2+} from the ER and causing an influx of extracellular Ca\textsuperscript{2+}. The elevated intracellular Ca\textsuperscript{2+} level enhanced parietal cell H\textsuperscript{+}-K\textsuperscript{-}-ATPase activity and subsequently gastric acid secre-
pathways. However, the distribution of CaSR in the colon crypt is controversial, and Chakrabarty et al indicated an increase of CaSR expression along the crypt axis, from the basal region to the top of the crypt (34,73). However, Ahearn et al revealed the converse conclusion in normal mucosa of colorectal adenoma patients (74). This difference in distribution may be caused by the different genera or the functional changes in CaSR expression during the development of colonic disease.

**Ca²⁺ and CaSR regulate intestinal secretion/absorption and fluid transport.** The intestine is the main organ of nutrient absorption and secretion that adjusts the flow of water and several ions (Na⁺, Cl⁻, K⁺, Ca²⁺) to maintain water and electrolyte homeostasis. Human intestinal mucosal epithelium contains a Ca²⁺ sensing mechanism. In the early 1980s, changes in extracellular Ca²⁺ as well as modulations in 1,25-dihydroxy vitamin D₃ levels were observed to regulate the absorption and/or secretion of Ca²⁺ in rat isolated colonic mucosa (75,76). CaSR participated in the regulation of intestinal secretion and absorption associated with Ca²⁺, organic nutrients and amino acids. Mace et al found that CaSR was involved in the L-amino acid stimulation of enteroendocrine (IEC) K-cell and L-cell activity in the rat small intestine (77). The intestinal brush border expresses CaSR, which helps to sense the presence of intraluminal calcium and modifies calcium transcellular and paracellular absorption by co-operating with the vitamin D system (78). The presence of CaSR provides a fundamental mechanism that intestinal cells use to detect and respond to Ca²⁺-related biologic behavior in intestinal secretion and absorption.

In addition, the CaSR is known to play a central role in intestinal fluid transport. It was reported that colonic CaSR is activated by Ca²⁺/spermine and that this activation could reverse the forskolin-stimulated net fluid secretion in isolated rat colonic crypts (79,80). The secretagogues such as forskolin or cholera toxin could induce fluid secretion by three important mechanisms, including the second message addition of cAMP and cGMP, the decrease in the activity of Na⁺-dependent fluid absorption ion channel sodium-hydrogen exchanger (NHE) (81,82), and an increase in the phosphorylation activation of Cl⁻-transport bumetanide-sensitive Na-K-2Cl cotransporter (NKCC1) by CFTR (83). CaSR was shown to reverse the generous fluid secretion by increasing the NHE activity, inhibiting CFTR-NKCC1 pathway and enhancing the cyclic nucleotide destruction to abrogate the addition of cAMP and cGMP (79,83,84). Thus, the presence of CaSR may have important implications for the prevention and treatment of certain diarrheal diseases. Moreover, Ca²⁺ and CaSR were required for polyamine (spermine > spermidine > putrescine) regulated fluid secretion. Cheng et al (85) observed in rat colonic crypts that spermine can not directly induce agonist response in the absence of Ca²⁺ and that the interaction of spermine with extracellular Ca²⁺ is able to enhance the Ca²⁺ sensitivity of the CaSR and modulate luminal or basolateral fluid.

**Figure 2. Summary of the known functions for the Ca²⁺-sensing receptor (CaSR) in the digestive system.**

**The role of CaSR in colonic cancer and drug resistance.** The consensus viewpoint is that CaSR as a tumor suppressor is down-regulated during colorectal tumorigenesis (86,87). The activation of CaSR promotes colon cancer epithelial cell differentiation and decreases cell growth. Thus, high Ca²⁺ dietary intake could reduce the risk of colorectal cancer development (88). According to a previous study, the mechanisms of CaSR that suppress colon cancer were relatively clear: the cell-cell intercellular adhesion protein and epithelial-mesenchymal transition important marker E-cadherin played key roles in the CaSR-related tumor-suppressing effects in colon cancer (34). On the one hand, the increase in E-cadherin stimulated by CaSR can interact with
β-catenin, an important protooncogene, which is the member of the Wnt pathway family that enhances the cell-cell and cell-matrix adhesion via the actin-based cytoskeleton (89). This may contribute to reducing the ability of cancer cells to move and invade surrounding tissues. On the other hand, the activation of CaSR can also prevent the nuclear translocation of β-catenin, thereby reducing β-catenin-TFAP complex formation and downregulating the c-myc and cyclin D1 expression. This inhibits cell proliferation and the defective Wnt pathway activation in colon cancer cell lines (34,90). In addition, CaSR has been reported to mediate non-canonical Wnt signaling (Wnt5a/Ror2) and to decrease the risk of colitis-associated colon cancer by suppressing NFκB activity and reducing inflammation TNFα secretion and TNFR1 expression (91). Thus, there is no doubt as to the anti-oncogenic role of CaSR. Interestingly, in 2013, Singh et al. found that CaSR-null colon cancer cells regained CaSR expression through the methylation and demethylation of the CaSR gene, followed by the concurrent reversal of stem cell markers, drug resistance and epithelial-mesenchymal transition (EMT) related transcription factors (92). Taken together, these data show that the inactivation of CaSR may serve as a key role in colon carcinogenesis.

In recent years, CaSR research has focused on the drug resistance of colon cancer chemotherapy. Activated CaSR can enhance the sensitivity of human colon carcinoma cells to mitomycin C (MMC) and fluorouracil (5-FU). Cancer cells with a high level of expression of thymidylate synthase (TS) and survivin are relatively resistant to the 5-FU (93). Nevertheless, NAD(P)H:quinone oxidoreductase 1 (NQO1) is a key enzyme involved in the bioreductive activation of MMC, and its reduced level is associated with MMC resistance in colon cancer cells. The activated CaSR could up-regulate the expression of NQO1 and downregulate the expression of both TS and survivin to promote cell apoptosis during chemotherapy. The conceivable mechanism is that CaSR activation suppresses the transcriptional activation of the survivin gene by inhibiting the β-catenin/Wnt signal pathway and decreasing the formation of the T-cell factor/β-catenin dimer (34). The dimer binding site is the survivin gene promoter (94,95). Moreover, CaSR can suppress c-Myc expression to negatively affect the transcription of the TS gene (96,97). In 2011, Liu et al. found that CaSR could act synergistically with the voltage-activated L-type Ca2+ channel (VGCC) blocker nifedipine to increase the release of Ca2+ from intracellular stores and enhance its sensitivity to 5-FU metabolism (98). The human multidrug resistance 1 (MDR1) gene and 5-FU metabolism (98).

Presently evidence demonstrates that the CaSR directly or indirectly regulates a variety of aspects of gastrointestinal physiological function and disease occurrence (Fig. 2). Recently, the function of CaSR in inflammation-associated digestive disease (such as esophagitis and colitis-associated colon cancer in humans) have been a direction of new research. CaSR as a new therapeutic target for treating digestive diseases has extensive clinical significance, further research should pay more attention to the application of scientific research for clinical applications.

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References


