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Multiple Roles for Cholinergic Signaling in Pancreatic Diseases

Cholinergic Signaling in Pancreatic Diseases

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Abstract

Cholinergic nerves are widely distributed throughout the human body and participate in various physiological activities, including sensory, motor, and visceral activities, through cholinergic signaling. Cholinergic signaling plays an important role in pancreatic exocrine secretion. A large number of studies have found that cholinergic signaling overstimulates pancreatic acinar cells through muscarinic receptors, participates in the onset of pancreatic diseases such as acute pancreatitis and chronic pancreatitis, and can also inhibit the progression of pancreatic cancer. However, cholinergic signaling plays a role in reducing pain and inflammation through nicotinic receptors, but enhances the proliferation and invasion of pancreatic tumor cells. This review focuses on the progression of cholinergic signaling and pancreatic diseases in recent years and reveals the role of cholinergic signaling in pancreatic diseases.

Core Tip: Cholinergic signaling participates in the physiological function of the pancreas and the pathological process of pancreatic diseases. Although the mechanism by which cholinergic signaling regulates pancreatic diseases is still unclear, an increasing number of studies have shown that cholinergic signaling plays a key role in the occurrence and development of pancreatic diseases. There are many animal models of pancreatitis that can be used to help us study the pathogenesis and pathophysiological process of pancreatitis. Among them, rodent models are most commonly used to study acute and chronic pancreatitis, but experimental pancreatitis is not necessarily the most relevant to human diseases. Due to the lack of an in-depth understanding of its pathogenesis, there is no effective prevention and treatment

strategy at resent. The mutant M3 receptor model provides an alternative method for the study of pancreatitis. The pancreas is an organ rich in nervous tissue and lies behind the peritoneum and is surrounded by many nerve plexus. Cholinergic signaling has different effects due to its action on different receptors, which leads to a complicated pathogenesis of pancreatic diseases such as acute pancreatitis, chronic pancreatitis, and pancreatic tumors. Reducing or enhancing the signal downstream of the receptor may provide a potential therapeutic target in the future.

INTRODUCTION

Acetylcholine is a neurotransmitter released by all cholinergic neurons that plays an important role in the peripheral and central nervous systems. The vagus nerve is the longest nerve in the human body and provides innervation to most organs, especially the organs of the digestive system. Cholinergic signaling released by vagus nerve activation mainly acts on cholinergic receptors. According to pharmacological properties, cholinergic receptors are divided into muscarinic receptors (M receptors) and nicotinic receptors (N receptors). Among them, M receptors are widely distributed in smooth muscles and glands and participate in the secretion of glands. M receptors are divided into 5 subtypes of M1-M5, among which M1, M3, and M5 receptors are coupled to Gq protein and M2 and M4 receptors are coupled to Gi protein[1, 2]. Each of these five subtypes of M receptors has a unique distribution pattern and is expressed in many areas of the central nervous system and peripheral tissues[1, 3-5]. N receptors are ligand-gated ion channels, a pentamer made up of 5 identical homologous subunits (including α1-10, β1-β5, γ, δ, and ε) and retain the potential to be activated by the appropriate agonist[6-8].

The pancreas is an important digestive organ of the human body and plays an important role in the digestion and absorption of food. Pancreatic diseases include acute pancreatitis, chronic pancreatitis, and pancreatic cancer. Recently, the incidence of pancreatic disease has been increasing year by year, posing a serious threat to human life and health[9-11]. Recent studies have found that cholinergic neuromodulation is

involved in the occurrence and development of pancreatic diseases[12-14]. This article reviews the relationship between the three main types of pancreatic diseases (acute pancreatitis, chronic pancreatitis, pancreatic cancer) and cholinergic signaling.

CHOLINERGIC SIGNALING AND PANCREATIC EXOCRINE FUNCTION

The exocrine system of the pancreas includes three different stages, namely, the cephalic stage, the stomach stage, and the intestinal stage[15]. The total amount of daily exocrine activity of the human pancreas is 1-2 L, and approximately 20% occurs in the cephalic stage, which is under the control of the vagus nerve[16-18]. Studies on humans and animals have shown that the cholinergic system regulates pancreatic exocrine secretion through the vagus nerve reflex. These reflexes originate in the dorsal motor nucleus of the vagus nerve in the medulla oblongata. The cranial preganglionic nerve fibers exit the vagus nerve and terminate at the intrapancreatic ganglia to form synapses through cholinergic preganglionic fibers[19, 20].

The intrapancreatic ganglion are the integration center of pancreatic exocrine secretion, and terminal axons from these ganglia innervate approximately every acinar artery. Although preganglionic neurotransmission is mediated by acetylcholine through nicotinic and muscarinic receptors, postpancreatic innervation can be mediated by a variety of neurotransmitters, including acetylcholine, which acts on muscarinic receptors in pancreatic acinar cells[21, 22]. Cholinergic agonists produce a pancreatic secretory response similar to that of cephalic stimulation, whereas cholinergic antagonists or resection of the vagus nerve can block the cephalic response. These results show that the acetylcholine released by the efferents of the vagus nerve is the primary mechanism by which sensory input leads to the regulation of pancreatic exocrine secretion[23-26]. The cholinergic system also plays an important role in the gastric and intestinal stages of pancreatic secretion regulation[27, 28], which are regulated by nerves and bodily fluids. In the intestinal phase, exocrine secretion of the pancreas is mainly regulated by cholecystokinin (CCK) and other gastrointestinal hormones. However, the cholinergic system also plays a role in the secretion of human pancreatic

enzymes stimulated by CCK[21, 29]. The postprandial physiological dose of CCK mainly acts on the afferent pathway of the vagus nerve in the gastric and duodenal mucosa and stimulates pancreatic exocrine secretion through the cholinergic efferent nerve[19].

Studies have found that human pancreatic acinar cells preferentially express the M3 receptor[30] and are highly expressed in acinar cells[31]. Acetylcholine acts on the M3 receptor and couples to the Gq protein to activate phospholipase C (PLC) and promote the release of intracellular calcium ions by initiating the phosphatidyl C-inositol triphosphate (IP3) cascade and promoting the secretion of pancreatic acinar cells[2, 32, 33]. According to drug blocking studies, the M3 receptor is a muscarinic receptor that stimulates exocrine secretion form the pancreas[34-36]. However, some studies have shown that the M1 receptor can also control pancreatic exocrine secretion[37, 38]. The inhibitory effect of drugs blocking the M1 receptor on the secretion of amylase in isolated pancreatic acinar cells was significantly greater than that of drugs blocking the M3 receptor[39]. Carbachol-induced amylase secretion was significantly impaired in the isolated acinars with M1 and M3 muscarinic receptor single knockout (KO) mice, and amylase secretion was eliminated in the acinar preparation of M1 and M3 muscarinic receptor double KO mice. Therefore, it is proposed that cholinergic signaling stimulates the secretion of pancreatic amylase and is mediated by the combination of M1 and M3 receptors[40].

CHOLINERGIC SIGNALING AND ACUTE PANCREATITIS

Acute pancreatitis (AP) is a common pancreatic disease in clinical practice. AP has a rapid onset and progress, and high morbidity and mortality worldwide[11]. There are many causes of AP, such as biliary, hypertriglyceridemia, and chronic alcohol consumption, among which biliary is the most common factor[41]. However, the specific mechanism underlying the cause of pancreatitis is not clear. When the human body is exposed to cholinergic agonists, such as scorpion stings or organophosphorus pesticide poisoning, some patients will develop acute pancreatitis. This is direct evidence that cholinergic signaling stimulation is associated with the occurrence of human acute

pancreatitis[42-45]. Organophosphorus pesticides can inhibit cholinesterase activity and cause a large amount of acetylcholine to accumulate in nerve endings. Similarly, scorpion toxin is a neurotoxic protein that can cause acute pancreatitis by activating the nerve pathway that releases acetylcholine[46, 47].

Pancreatic duct ligation in the rat model is often used to simulate clinical acute pancreatitis caused by gallstone obstruction[48], but the severity of experimental pancreatitis is low except when used in the possum model. However, some researchers have found in a pancreatic duct ligation rat model that cholinergic signaling stimulation can aggravate acute pancreatitis inflammation, suggesting that cholinergic signaling may be involved in the pathogenesis of acute pancreatitis caused by cholangiopancreatic duct obstruction[49]. This may be due to an increase in M3 receptor expression induced by rat pancreatic duct ligation, which amplifies the overstimulation of cholinergic signaling on acinar cells and aggravates the intracellular stress response[50]. Before the first clinical onset of acute alcoholic pancreatitis, patients usually have a history of alcohol abuse for many years. Chronic alcohol intake can affect the exocrine regulation of the pancreas by interfering with the cholinergic and trypsin pathways[51]. Long-term use of ethanol feeding can significantly reduce pancreatic acetylcholinesterase activity in rats, while the expression level of pancreatic cholinergic M receptors has not changed, which increases the level and duration of acetylcholine in the pancreas and leads to excessive cholinergic signaling stimulation and damage to pancreatic acinar cells[52, 53]. Ethanol-treated pancreatic acinar cells can aggravate the pancreatic injury response caused by the cholinergic agonist carbachol, and its effect may be mediated by protein kinase C (PKC) downstream signaling of cholinergic receptors[54]. The cholinergic receptor antagonist atropine can improve pancreatitis induced by the combination of alcohol and cerulein suggesting that the cholinergic signaling pathway is involved in the pathogenesis of pancreatitis[52]. Therefore, acetylcholine may play a key role in the pathogenesis of acute alcoholic pancreatitis[12].

The M3 receptor is highly expressed in human pancreatic acinar cells[31]. Wan *et al* used chemical genetic technology, in which designer receptors are exclusively activated

by designer drugs (DREADDs), to express mutant M3 receptors in mouse acinar cells, causing them to lose their response to acetylcholine but can be activated by the specific drug clozapine-N-oxide (CNO). CNO can induce acute pancreatitis in mutant M3 receptor mice and cause more extensive acinar cell necrosis and inflammation. In addition, the use of M3 receptor antagonists can improve the severity of AP induced by cerulein in wild-type mice **(Figure1)**[55].

These results indicate that the activation of the M3 receptor by cholinergic nerve terminals releasing acetylcholine may be one of the pathogeneses of acute pancreatitis. Muscarinic receptor agonists stimulate the activation of trypsinogen and NF-κB, which are two key signaling pathways in the pathogenesis of pancreatitis[56]. Reducing inflammation has always been a major goal in the treatment of acute pancreatitis, and many anti-inflammatory drugs have shown beneficial responses in experimental pancreatitis[57]. However, due to the lack of an in-depth understanding of its pathogenesis, there is currently no effective prevention and treatment strategy[58]. Through in-depth research on the cholinergic signaling pathway, it may be possible to block cholinergic signaling early to treat acute pancreatitis, especially in the prevention of pancreatitis after endoscopic retrograde cholangiopancreatography (ERCP).

CHOLINERGIC SIGNALING AND CHRONIC PANCREATITIS

Chronic pancreatitis (CP) is characterized by chronic inflammation and fibrosis of the pancreas caused by multiple factors. The incidence and prevalence of CP are increasing each year, but there is no current specific treatment[10]. The most common causes of chronic pancreatitis are excessive drinking, smoking or genetic mutations[59]. Alcohol is considered to be one of the main risk factors for chronic pancreatitis. A total of 40-70% of chronic pancreatitis patients become sick due to excessive alcohol consumption[10]. At the same time, excessive alcohol consumption increases the risk of pancreatic cancer in individuals[60].

Recurrent episodes of acute pancreatitis have been associated with progression of chronic pancreatitis, which is more common in alcoholics. It has been reported that a

certain extent of chronic pancreatic damage was already present at the time of acute pancreatitis episodes[41]. The dose-response relationship between alcohol consumption for AP and CP is linear in men[61]. Alcohol-induced pancreatitis may be caused by alcohol-induced increased viscosity of pancreatic secretions, which blocks the pancreatic duct, and by premature activation of trypsinogen in acinar cells[62].

The hypertonicity of intrapancreatic cholinergic neurons caused by chronic alcoholism may be involved in the pathogenesis of chronic pancreatitis[13]. In CP patients, ethanol can cause excessive sensitivity of the pancreatic parasympathetic nerve pathways after a meal[63]. This may be due to chronic alcoholism interrupting the autonomic nerve suppression reflex, leading to the decentralization of the intermediate autonomic nerve (located in the gastric antrum and duodenum) and the intrapancreatic ganglia. Due to an increase in the activity of these autonomic nerves, cholinergic signaling in the pancreas increases, resulting in excessive protein secretion and obstruction of pancreatic juice flow[64, 65]. Histopathological analysis of pancreatic tissue samples from patients with chronic pancreatitis (including alcoholic pancreatitis) demonstrated that the density of cholinergic fibers in the pancreas of patients with chronic pancreatitis was slightly increased compared with normal pancreatic tissue samples[66]. Therefore, the impaired interaction between cholinergic signaling and their receptors on pancreatic acinar cells may be a mechanism of the pathogenesis of alcoholic pancreatitis.

Compared with acute pancreatitis, few chronic pancreatitis models use injury mechanisms that may be related to the pathogenesis of human diseases, and the clinical relevance of the pathogenesis of most chronic pancreatitis models is unclear[48]. However, Wan *et al* used a mutant M3 receptor mouse-induced CP model and observed typical CP features, such as extensive chronic inflammation, fibrosis, adipose tissue infiltration, and pancreatic atrophy **(Figure1)**[55]. The use of this M3 receptor model may increase our understanding of the pathophysiological process of human CP and identify specific treatments.

Long-term and recurrent pain is a common characteristic of chronic pancreatitis[10, 67]. A large number of studies have shown that cholinergic nerves have a significant analgesic effect on chronic neuropathic pain, inflammatory pain, and visceral pain[68-70]. Choline transporter (CHT1) is considered to be the rate-limiting step of neuronal acetylcholine synthesis and is essential for the effective recovery of acetylcholine[71, 72]. CHT1 is upregulated in CP-induced pain models. CHT1 specific inhibitor, hemicholinium-3 (HC-3), can significantly enhance CP-induced hyperalgesia and reduce the amount of acetylcholine in the dorsal root ganglia of the pancreas in a dose-dependent manner[73]. Further research found that acetylcholine reduces pain and inflammation through α7 nicotinic acetylcholine receptors (α7 nAChRs)[70, 74-77]. The activation of α7 nAChRs enhances the autophagic flux of acinar cells mediated by the transcription factor EB (TFEB) pathway and promotes lysosomal degradation to inhibit acinar cell damage, thereby protecting experimental pancreatitis. It has been suggested that cholinergic signaling activation of endogenous α7 nAChRs in the pancreas may be an endogenous protective mechanism in the process of pancreatitis[78]. Therefore, the use of α7 nAChRs to develop analgesic and anti-inflammatory drugs will be a promising target, especially for the treatment of chronic pancreatitis.

CHOLINERGIC SIGNALING AND PANCREATIC CANCER

Pancreatic cancer (PCa) is a gastrointestinal tumor with a poor prognosis, and more than 90% of pancreatic cancers are pancreatic ductal adenocarcinoma (PDAC). According to reports, the 5-year survival rate for pancreatic cancer patients in the United States from 2009 to 2015 was only 9%[79]. With the continuous increase in morbidity and mortality, pancreatic cancer is expected to become the second leading cause of cancer-related deaths by 2030[80].

Chronic pancreatitis has been identified as a risk factor for pancreatic cancer[81, 82]. A meta-analysis study found that chronic pancreatitis increases the risk of pancreatic cancer, five years after diagnosis of chronic pancreatitis in patients increased the risk of pancreatic cancer nearly eight-fold[83]. Pain is a characteristic feature of chronic

pancreatitis and pancreatic cancer. Studies have found that increased nerve fiber density and hypertrophy are typical features of CP and PCa, and this pathological change appears to enhance and produce pancreatic neuropathic pain[84]. In vitro, myenteric plexus and dorsal root ganglia neurons are isolated from neonatal rats with CP and PCa tissue extracts, which exhibit strong neurite outgrowth, more complex branching patterns, and somatic hypertrophy, suggesting that the intrapancreatic microenvironment in CP and PCa appears to be a key factor in the generation of pancreatic neuropathy and neural plasticity[85].

In-depth research on the tumor microenvironment found that perineural invasion is an important feature of pancreatic cancer, which can lead to local tumor recurrence and poor prognosis[86]. As the degree of invasion increases, the survival rate of pancreatic cancer patients is significantly reduced[87]. However, cholinergic signaling appears to play a tumor-suppressing role in pancreatic cancer[14, 88]. Regarding the relationship between heart rate variability (HRV) as an indicator of vagus nerve activity and the overall survival of patients with advanced pancreatic cancer, it was found that higher vagus nerve activity represented by a higher initial HRV was significantly associated with a lower risk of death from pancreatic cancer and was not affected by confounding factors such as age and cancer treatment[89]. The activation of M receptors by cholinergic signals can inhibit the progression of pancreatic tumors. Inhibition of the downstream EGFR/MAPK and PI3K/AKT signaling pathways of pancreatic cancer cells through the M1 receptor signaling pathway inhibits tumor stem cells, CD11b$^+$ cells, TNF-α levels, and liver metastasis[14].

However, M3 receptor expression seems to be a biomarker for the poor prognosis of PDAC. Compared with patients with low M3 expression, patients with high M3 expression have a worse prognosis and shorter survival time, but the study did not find a statistically significant relationship between M3 receptor expression and peripheral nerve infiltration in pancreatic cancer[90]. The presence of nonneuronal acetylcholine secreted by fibroblasts and pancreatic stellate cells (PSCs) in the microenvironment of pancreatic tumors activates the M3 receptor, leading to further tumor progression[91].

Oxidative stress and inflammatory signaling contribute to the development of pancreatitis. Español *et al* reported that long-term inflammatory stimulation by LPS plus IFN-γ could induce muscarinic receptor expression which mainly involved the M3 and M5 receptors, and also could up-regulate the expression of NOS and COX-2 to enhance the effect of carbachol on NO and PGE_2 production[92]. Transgenic overexpression of COX-2 in the pancreas induces chronic pancreatitis and the formation of preinvasive ductal tumors[93, 94]. Targeting muscarinic receptors as promising therapeutic targets for pancreatic inflammation could prevent the transformation of chronic pancreatitis to pancreatic cancer.

The increase in acetylcholine levels by nAChRs can inhibit histone deacetylase 1 (HDAC1)-mediated CCL5 chemokines, weakening the ability of PDAC cells to recruit $CD8^+$ T cells and directly inhibit $CD8^+$ T cells from producing IFN-γ, which is conducive to Th2 differentiation, thereby promoting tumor growth[86]. Smoking is one of the main causes of pancreatic cancer[95], and approximately 21% of pancreatic cancer deaths are attributed to smoking[96]. Studies have shown that nicotine (nAChR agonist) can increase the proliferation activity and self-renewal ability of pancreatic cancer stem cells by activating the sonic hedgehog signaling pathway[97]. α7 nAChRs have also been shown to upregulate mucin-4 (MUC4) by coactivating the JAK2/STAT3 downstream signaling cascade with the MEK/ERK1/2 pathway, thereby increasing the migration and invasion capabilities of pancreatic cancer cells[98]. Because the pancreas is widely innervated by many different neurons, the relationship between pancreatic cancer and the nervous system is complicated[99]. Cholinergic signaling produces different effects based on the different receptor mechanisms involved and may become a potential therapeutic target in the future for pancreatic cancer.

CONCLUSION

Cholinergic signaling participates in the physiological function of the pancreas and the pathological process of pancreatic diseases. Although the mechanism by which cholinergic signaling regulates pancreatic diseases is still unclear, an increasing number

of studies have shown that cholinergic signaling plays a key role in the occurrence and development of pancreatic diseases. There are many animal models of pancreatitis that can be used to help us study the pathogenesis and pathophysiological process of pancreatitis. Among them, rodent models are most commonly used to study acute and chronic pancreatitis, but experimental pancreatitis is not necessarily the most relevant to human diseases[48]. Due to the lack of an in-depth understanding of its pathogenesis, there is no effective prevention and treatment strategy at present. Activation of the M3 receptor may be one of the causes of pancreatitis since muscarinic receptors are widely expressed in various glands and smooth muscles throughout the body. The mutant M3 receptor model provides an alternative method for the study of pancreatitis[55]. The pancreas is an organ rich in nervous tissue and lies behind the peritoneum and is surrounded by many nerve plexus. Cholinergic signaling has different effects due to its action on different receptors, which leads to a complicated pathogenesis of pancreatic diseases such as acute pancreatitis, chronic pancreatitis, and pancreatic tumors **(Table1)**. Reducing or enhancing the signal downstream of the receptor may provide a potential therapeutic target in the future.

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