Role of Neurogenic Inflammation in Pancreatitis and Pancreatic Pain

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Key Words

Inflammatory pain · Pancreatitis · Visceral pain

Abstract

Pain arising from pancreatic diseases can become chronic and difficult to treat. There is a paucity of knowledge regarding the mechanisms that sensitize neural pathways that transmit noxious information from visceral organs. In this review, neurogenic inflammation is presented as a possible amplifier of the noxious signal from peripheral organs including the pancreas. The nerve pathways that transmit pancreatic pain are also reviewed as a conduit of the amplified signals. It is likely that components of these visceral pain pathways can also be sensitized after neurogenic inflammation.

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Introduction

Pancreatitis is a clinical condition in which the main pathology is inflammation of the pancreatic tissue. The duration of the disease process serves as the main indicator for classification of this condition. Acute pancreatitis (AC) is a mild, self-limited condition, typically present with elevated serum levels of pancreatic enzymes [1]. The incidence of acute pancreatitis has been increasing over recent years [2]. Most cases are secondary to biliary disease and alcohol abuse [3]. Chronic pancreatitis (CP) of-

ten represents progressive destruction of the pancreas by repeated flare-ups of silent or symptomatic acute pancreatitis [4]. During the inflammatory disease process there is a slow replacement of viable tissue by fibrotic tissue and alterations in the pancreatic nerves around the areas of inflammation [5]. The clinical incidence of chronic pancreatitis is about 4 per 100,000 inhabitants [6] but this has been rising over the last 20 years.

The symptom that most often brings the patient with pancreatitis to the clinic is abdominal pain. Pain is the only sensation that can be elicited from the pancreas [7]. Pancreatic pain is usually sensed as a severe epigastric discomfort that may radiate to the right and/or left upper quadrant and to the back [8]. Patients describe pancreatic pain as ‘stabbing’, ‘burning’, and ‘boring’. There is limited functional information concerning pain in the pancreas; some inferences must be drawn from studies on the generation of pain in other viscera [9, 10]. The etiology of pancreatic pain is a matter of controversy, ranging from neurogenic inflammation to increased pancreatic pressure. Nonetheless it is agreed that the final common pathway of any pathological process that produces pain in the pancreas is the nerve supply to this organ [4].

Neurogenic inflammation is the process by which substances released from sensory nerve terminals produce inflammation in their target tissue. This inflammation includes arteriolar vasodilation and plasma extravasation accompanied by degranulation of mast cells [11]. The principal trigger of neurogenic inflammation is the acti-

vation of primary afferents giving rise to dorsal root reflexes in the spinal cord [12]. The ensuing inflammation supported and perpetuated by the enhanced excitability in the spinal cord contributes to the amplification of pain signals from the periphery.

The purpose of this review is to give an overview of the currently known mechanisms of pancreatic pain transmission and evidence for the establishment of neurogenic inflammation as a possible mechanism that initiates the development of persistent transmission of pain signals from the pancreas.

Pancreatic Pain

Most of the present knowledge about pancreatic pain has come from clinical reports. Blocking or sectioning the sympathetic innervation, but not the vagus, is highly effective in managing pancreatic pain; thus visceral afferent fibers traveling with the sympathetic nerves must transmit nociceptive information from the pancreas [13–18]. These sympathetic nerves pass through the celiac plexus and continue toward the central nervous system through the greater splanchnic nerves. The greater splanchnic nerves pass obliquely toward the spinal column to join the sympathetic trunk and without synapsing, the visceral afferent fibers enter the spinal cord through the spinal nerves at thoracic levels five to ten. Their cell bodies lie in the thoracic dorsal root ganglia. Signals carried by the afferent nerves serving the pancreas normally do not come to the level of consciousness. When they do, they are perceived as pain. Ascending tracts in the spinal cord carry the nerve impulses to regions of the brain, where, under the appropriate conditions, the encoded signals are perceived as pain.

It has been known for some time that limited information about visceral pain is transmitted to higher brain centers by the spinothalamic tract [19–25], the spinoreticular tract [26], the spinoparabrachial tract [27, 28] and pathways that project directly from the spinal cord to forebrain structures which include the amygdala and the septal nuclei [29, 30]. Recently, it has been demonstrated that a portion of the postsynaptic dorsal column pathway (PSDC), which primarily transmits discriminative tactile, vibratory and proprioceptive sensation [31–33] can transmit nociceptive signals from visceral structures, including the pancreas. Clinical evidence showed that pelvic cancer pain can be suppressed by limited neurosurgical lesions of the midline of the dorsal column pathway [34–38]. The pain was completely relieved after this sim-

ple neurosurgical procedure, and large doses of morphine were no longer needed. The patients' vibratory, light touch, and proprioceptive sensation were unchanged. Cancer pain from several abdominal organs has been relieved similarly by this type of surgery [39]. Retrograde and anterograde anatomical tract tracing studies of visceral input demonstrate a neuronal pathway which arises from cell bodies situated around the central canal of the spinal cord and travels in the midline of the dorsal column to the dorsal column nuclei [40–42]. Compelling electrophysiological evidence indicates that this PSDC pathway transmits nociceptive information from the colon [43–46] and the duodenum [47]. Recently it has been demonstrated that nociceptive information from the pancreas is also transmitted by the PSDC pathway. Lesions that interrupt this pathway abolish the behavioral manifestations of visceral pain brought about by experimental pancreatic inflammation [48]. Lesions of the DC pathway also interrupt the transmission of nociceptive signals to the thalamus from the inflamed pancreas [49]. In addition to the dorsal column nuclei, the nociceptive signals from the pancreas also project to other supraspinal sensory structures such as the medial thalamus, raphe magnus, parabrachial nuclei and the periaqueductal gray [42, 50]. Thus, nociceptive transmission from the diseased pancreas reaches levels of consciousness, and is interpreted as pain, through distinct visceral pain pathways.

The origin of the pain signals from the pancreas is a matter of great discussion. Surgical drainage procedures used to treat pancreatic pain in patients with chronic pancreatitis are based on the assumption that increased pressure within the ductal system and the pancreatic parenchyma is responsible for the generation of pain. The pain relief resulted from the drainage procedure would appear to be related to a decrease in the pressure. Increased pressure causes changes in blood flow and pH [51] in experimental animals. Ischemia is a known stimulus of pain, and H^+ is also important in this regard [52].

The nerves that innervate the pancreas are mainly unmyelinated. In tissue from patients with chronic pancreatitis, foci of chronic inflammatory cells frequently are concentrated around nerves [5]. Electron-microscopic examination of pancreatic tissue from patients experiencing pain because of chronic pancreatitis shows that the chronic inflammation has damaged the nerves. The protective barrier normally provided by the perineurium is no longer intact and the nerve is edematous [5]. Inflammatory cells may invade the inner part of the nerve. Loss of the barrier makes the inner part of the nerve susceptible to factors released from the inflammatory cells (neurogenic

inflammation, see next section) or from damaged acinar cells. These changes may initiate impulses in the affected fibers which are essential for the initiation of neurogenic inflammation.

Neurogenic Inflammation

At the turn of the 20th century, it was first demonstrated that the activation of dorsal root ganglion neurons induced vasodilation [53], suggesting that neurons not only conduct afferent information to the spinal cord, but also subserve an efferent function. The small diameter sensory neurons that are activated by capsaicin (the vanilloid found in hot peppers) are particularly important in neurogenic inflammation [54]. Intradermal injection of capsaicin rapidly produces cutaneous inflammation, which does not occur if the skin is deprived of its sensory innervation [55–57]. Thus, sensory innervation is essential for the production of neurogenic inflammation. Activation of the sensory afferents can be triggered by a variety of substances including resiniferatoxin, mustard oil, bradykinin, arachidonic acid, xylene, and acrolein, as well as by heat, ischemia, or protons [58–60]. Some of these substances are produced during a typical inflammatory response ('the inflammatory soup'), which leads to the notion that once an inflammatory response has begun, it can be perpetuated by feed forward mechanisms that results in the continual activation of the sensory primary afferent [61]. Activated primary afferents release a variety of substances in their peripheral tissue targets. Among these, the neuropeptides substance P (SP) and calcitonin gene related peptide (CGRP) have been extensively studied. Substance P released into peripheral tissues causes plasma extravasation [62–68]. This extravasation is caused by SP acting on NK-1 receptors [69]. The plasma extravasation that follows sensory stimulation is prevented by NK-1 antagonists [70–74]. The actions of SP are for the most part vasoconstrictive (endothelial cell contraction giving rise to plasma extravasation). Nonetheless there are reports that SP can act as a vasodilator in part by stimulating the release of histamine from mast cells [75–78] or by direct action that does not involve the release of histamine. Antagonists for the NK-1 receptor can also abolish the SP-produced vasodilation [79, 80]. Calcitonin gene related peptide is a potent vasodilator that in low doses causes vasodilation by direct action [81–83]. The vasodilatory action of CGRP can be attenuated by the CGRP receptor antagonist CGRP_{8–37} [84] which also inhibits edema formation produced by stimulation of a

cutaneous nerve, suggesting that the vasodilation resulting from the release of CGRP contributes to edema formation.

The release of substances like SP and CGRP contribute to the reverberation of the inflammatory cascade in the affected tissue. Substance P can act to enhance release of proinflammatory mediators into the peripheral tissue. For example SP can increase production and release of prostaglandin E₂ [85], release of lysosomal enzymes [86] and release of interleukin 1 and the neutrophil chemoattractant interleukin 6 [87]. During inflammation, invading macrophages express NK-1 receptors [88, 89] suggesting that SP in peripheral tissues can perpetuate the inflammatory cascade. Many of these released substances can on their own sensitize neurons and enhance transmitter release, creating a reverberating circuit. For example, bradykinin can bind to the B₂ receptor on sensory neurons resulting in the activation of phospholipase C (PLC) giving rise to the second messengers, inositol 1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol (DAG). This classical pathway increases intracellular calcium concentration and activates various isoforms of PKC [90]. Activation of PKC can potentiate neuropeptide release, as demonstrated by direct activation of the enzyme with phorbol ester on cultured neurons [91, 92]. It is possible that activation of PKC by bradykinin causes depolarization of afferent fibers. Other compounds act to sensitize sensory neurons by lowering the threshold for firing [93]. For example, the prostaglandins released into peripheral tissue and spinal cord during tissue injury and inflammation [94] lower the firing threshold of neurons [95]. Other substances that can also induce neuronal sensitization are ATP and bradykinin [96, 97]. Finally, accumulating evidence indicates that the cytokines interleukin-1 β , interleukin-6 and tumor necrosis factor- α can increase the heat or capsaicin-evoked release of SP and CGRP in neuronal preparations [98, 99]. Long-term exposure to TNF- α also increases capsaicin currents in isolated neurons [100]. The transduction cascades that mediate cytokine sensitization actions on sensory neurons remain to be fully characterized. The actions of cytokine may be mediated in part by the release of prostaglandin, as inhibitors to COX2 can attenuate the sensitization process by cytokines [101]. The sensitizing actions of the proinflammatory prostaglandins PGE₂ and PGI₂ are mediated through the cAMP pathway [102]. Exposing sensory terminal endings to PGE₂ or PGI₂ at concentrations that can sensitize neurons will increase the production of cAMP [103, 104]. The increase in peptide release produced by prostaglandins is mimicked by forskolin, membrane-permeable

cAMP analogs, or cholera toxin. Compounds that inhibit cAMP-dependent phosphorylation block the prostaglandin-induced sensitization [105]. Other second messenger pathways that have been implicated in the processing of neurogenic inflammation are the PKC pathway and the mitogen-activated protein (MAP) kinase pathway. The PKC pathway can be activated by ATP and bradykinin (see above), while the MAP kinase pathways can be activated by the binding of nerve growth factor (NGF) to its receptor TrkA on peptidergic sensory neuronal endings resulting in an increase in the release of neuropeptides [106].

Neurogenic inflammation has been described in numerous tissues, including the skin, joints, eye, middle ear, dura mater and in the respiratory, genitourinary, reproductive and digestive systems. One of the best-described paradigms of neurogenic inflammation is experimental inflammation of a joint. The injection of kaolin and carrageenan into the capsule of the knee joint is a very useful model of arthritis in monkeys [107]. In this model, the primary afferent fibers that supply the knee joint become much more excitable than normal. Peripheral and central changes that occur following the initiation of inflammation by injection of kaolin and carrageenan into the knee joints of rats have also been described [108]. One of the most important observations using this model is that application of either the non-NMDA receptor antagonist CNQX or the NMDA receptor antagonist AP7 by microdialysis into the dorsal horn blocked the release of excitatory amino acids brought about by inflammation of the joint. Interestingly, the swelling of the joint and the temperature rise are reduced significantly by CNQX, but not by AP7 administration [109–111]. This evidence shows how activation of spinal cord circuits can affect events in peripheral tissues. The effects observed are mediated through one of several neural pathways that connect the knee joint and the spinal cord. The involvement of the sympathetic nerves was ruled out by experiments in which surgical and chemical sympathectomy failed to reduce the amount of joint swelling [112]. The swelling also occurs when the animals are deeply anesthetized and thus have little or no muscle tone ruling out a contribution of muscle contraction. This leaves joint afferents as the most likely element involved in the central mechanism for promoting joint swelling. Experiments to validate this hypothesis recorded antidromic afferent nerve activity, referred to as dorsal root reflexes, from the medial articular nerve (MAN). The dorsal root reflexes are elicited by mechanical stimulation around the inflamed knee joint [113]. In another series of experiments recordings were

made in rats from the MAN on the side contralateral to that of an injection of incomplete Freund's adjuvant into one knee joint [114]. Dorsal root reflexes were again observed, and local application of capsaicin to the MAN eliminated the dorsal root reflex activity. It was concluded that part of this activity is conveyed by capsaicin-sensitive articular afferents.

Antidromic stimulation of articular C fiber afferents has been shown to result in plasma extravasation in the knee joint [115]. Furthermore, denervation of the knee joint and administration of an SP receptor antagonist can reduce carrageenan-induced arthritis [116]. The role of CGRP in joint inflammation is not as clear as that of SP and other neurokinins, although vasodilator responses to both peptides have been shown to increase during carrageenan-induced arthritis [112, 117]. In summary, it seems likely that dorsal root reflexes that develop in fine afferents during experimental arthritis results in the release of peptides such as SP and CGRP into the joint initiating the process of neurogenic inflammation.

Neurogenic Inflammation in Pancreatitis and Its Relationship to Pain

In visceral tissues, neurogenic inflammation may not only be a pathophysiological mechanism leading to disease, but forms part of the tissue response to injury. Neurogenic inflammation seems to be an adaptive response, promoting rapid increases in tissue substances, activating cells for local defense and enhancing fluid transport to isolate and dilute bacteria and toxins. However, this process may become maladaptive as it has been demonstrated in asthma, arthritis and migraine [118] and has been suggested also in the development of interstitial cystitis [119].

In visceral pain conditions, neurogenic inflammation seems to be a factor in the mechanism of referred pain [120]. For example, pain from myocardial infarction may sometimes induce a left scapulohumeral periartthritis, an inflammatory condition in the referred zone [121]. Evidence for neurogenic inflammation in the somatic referred zone triggered by inflammation of a viscous has been demonstrated in an animal model of uterine pain in the rat [122]. Inflammation of the uterus resulted in neurogenic plasma extravasation in the skin over the abdomen, groin, lower back and perineal areas. In the pancreas, recent investigations have implicated the involvement of neurogenic inflammation in the pathophysiology of pancreatitis. The administration of SP to mice stimu-

lates plasma extravasation from postcapillary venules in the pancreas and this effect is blocked by antagonists to the NK-1 receptor [123]. Furthermore it has been shown that genetic deletion of the NK-1 receptor in mice reduces the severity of experimental-induced pancreatitis [124]. These findings show that SP is an important mediator of pancreatic inflammation. SP is likely to be released by nociceptive c fibers during pancreatitis because denervation by capsaicin reduces the severity of experimental pancreatitis [125]. The vanilloid receptor VR1 mediates SP release in the pancreas during experimental pancreatitis [126]. Receptors for SP have been found in acinar cells in the guinea pig where they act to stimulate secretion of pancreatic enzymes [127–129]. Likewise, SP can act on endothelial cell in the pancreas to produce edema [130] and can also produce its effects by activating of other inflammatory cells such as neutrophilic or mast cells [131, 132].

The previous evidence points to a role of SP as a pro-inflammatory mediator during pancreatitis, but its role as a mediator of pain and of neurogenic inflammation is still relatively unknown. The lack of a good animal model of pancreatic pain has hindered the investigation of this role. Recently, a rat model of pancreatic pain which is reliable and reproducible has been developed [133]. This model mimics the human condition by presenting signs of referred pain and responsiveness to morphine. Using this model, an antagonist to the NK-1 receptor attenuated the pain behavior seen in this model [134]. Also in this same study, NK-1 receptors were upregulated in the pancreas of animal with pancreatitis. The mechanism behind these results remains to be elucidated, but SP through

the NK-1 receptors can produce plasma extravasation in the pancreas (see above) and in another model plasma extravasation has been reported [130]. In the previous section we have discussed the proinflammatory action of SP by activating proinflammatory cytokines. These cytokines can produce hyperalgesia [101] and therefore could be one of the mechanisms generating pancreatic pain. Indeed, expression of SP and IL-8 in pancreatitis are correlated [135] and the upregulation of the NK-1 receptor in the pancreas is correlated with reports of pain levels in pancreatitis [136].

Concluding Remarks

Pancreatic pain is usually very difficult to treat with conventional analgesics. It is of great importance to elucidate the mechanisms of pain during pancreatitis using appropriate models that mimic the human condition with the hope of identifying new therapeutic targets. We now know the pathways by which pancreatic pain is transmitted to higher brain centers. The possibilities of neurosurgical interventions are implicit here, but it is a proposition that likely should be reserved for cases of pancreatic cancer where the pain is intractable and/or the quality of life is very poor. Other targets have emerged as mechanisms of inflammation in the pancreas itself as an engine of reverberating activation of pancreatic nerves and subsequent neurogenic inflammation that can sensitize sensory neurons in the periphery and perpetuate a circuit that leads to persistent pain conditions.

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