MINIREVIEW

Pathophysiological Effects of Nicotine on the Pancreas (44284)

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Nicotine, a major component of tobacco and cigarette smoking is an addictive agent and has been characterized as a drug of abuse by the US Surgeon General (1–3). The economic burden due to abuse of this drug is substantial because of the well-documented pathophysiological effects of nicotine on organs in the cardiovascular, respiratory, hepatic, renal, and nervous systems (3).

Association of nicotine through cigarette smoking with the increased incidence of pancreatitis and pancreatic cancer has also been reported (4–18). A survey on the association between cigarette smoking and pancreatic cancer showed that cigarette smokers had a significant 70% higher risk of pancreatic cancer in comparison to nonsmokers (8–16). When compared with nonsmokers, subjects who smoke filtered cigarettes had a 50% elevated risk. The proportion of pancreatic cancer attributable to cigarette smoking was 29% in blacks and 26% in whites (10). Most of the data that link cigarette smoke/nicotine to pancreatic diseases were gathered in humans. Recent studies with animals have also shown that nicotine or its metabolites could induce pathological and functional changes in the pancreas (17–25). This review will present and discuss the current understanding of the pathophysiology induced by nicotine in the exocrine pancreas and the possible mechanism of action of nicotine.

Effects of Nicotine and its Metabolites on the Structural and Functional Changes of the Exocrine Pancreas

Cotinine and nornicotine are natural metabolites of nicotine. About 80%–90% of the dose of nicotine consumed can be accounted for in human urinary metabolites. In rodents, hepatic nicotine metabolism is found to involve cytochrome p450s that catalyze the first step of this pathway (26, 27). It has been demonstrated that pancreatitis could be induced in mice fed a caerulein and choline-deficient ethionine (CDE) supplemented diet (28–33). The major histopathological changes noted in these animals included cytoplasmic vacuolation, cellular interstitial edema, and cellular necrosis with pyknotic nuclei and karyorrhexis. The appearance of cytoplasmic vacuoles in the exocrine pancreas was considered an early pathological marker of pancreatic injury (33). The vacuoles were found to contain digestive and lysosomal enzymes (34–36), and upon activation, they promoted degenerative changes in the pancreas (36–37). Exposure of animals to nicotine has been shown to induce morphological changes in the pancreas similar to those induced by caerulein and CDE diets (19, 20, 24, 25). It is, however, not clear whether the changes induced by nicotine also involve activation of proenzymes to active enzymes leading to further tissue destruction.

The precise pathological effects of nicotine and its metabolites on the exocrine pancreas are still unclear. The dose-response and time-course effect of nicotine-induced pathology (e.g., edematous, vacuolar, pyknotic changes as well as alterations in mitochondria and other organelles) also need to be examined and further characterized.

Effects of Nicotine on Gastrointestinal Function

Gastrointestinal secretions in humans are influenced by cigarette smoking (38), and evidence suggests that nicotine has a direct effect on pancreatic secretions (21–25, 39–41). It has been shown that when rabbits were exposed to nicotine, a significant decrease in secretion of duodenal bicarbonate occurred (41). A decreased responsiveness to secretagogues in the pancreas was also found in rats exposed to nicotine (20, 21, 24, 25). Exposure of isolated pancreatic acini to nicotine *in vitro* enhanced secretion of hydrolases

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and newly synthesized proteins (23). These studies suggest that nicotine and its metabolites have a definitive effect on exocrine pancreatic secretions.

Chronic cigarette smoking has been linked directly to pulmonary emphysema with correlated reduction in endogenous antiproteases (42). It has been shown that changes in the basal serum levels of the gastrointestinal hormones CCK (43–46), or serum enzymes such as amylase and lipase (47–49), are associated with pancreatic injury (47–53). It is of interest to note that when subjected to a single injection of secretin, there was a significantly higher serum concentration of pancreatic digestive enzymes in smokers than in nonsmoking controls (52), suggesting that some form of pancreatic injury occurred in smokers.

Pathophysiological Effects and Mechanism of Action of Nicotine on the Exocrine Pancreas

Gastrointestinal hormones such as CCK, carbachol, and secretin can be used as ligands for pancreatic receptors to determine pancreatic function since these peptides stimulate exocrine pancreatic secretion through specific receptor mediated pathways (44–47, 53). Ligand receptor interactions induce the release of amylase through complex signal transduction events and can be determined colorimetrically.

An isolated cell model has been used to determine the mechanism of the underlying pathologic changes in the acinar cells (47, 53–59). Acinar cells are programmed to respond to a given stimulus with a coordinated release of secretory granule content. This response indicates the existence of intracellular messengers which in turn transduce the external signal for an increased rate of vesicle membrane fusion and secretory action. The intracellular messengers play a regulatory role in exocytotic secretion and are the key factors in signal transduction pathways (47, 53, 59, 60). Two major classes of receptors have been identified in acinar cells based on their responses to different agonists: those coupled to mobilization of cellular calcium (CCK, bombesin, carbachol) and those coupled to activation of adenylate cyclase (secretin, vasoactive intestinal peptide) (53, 59, 60).

A schematic diagram describing the multiple signal transduction pathways in an isolated acinar cell model is shown in Figure 1. Preliminary data obtained in our own laboratory suggest that these pathways are directly or indirectly involved in the inhibition of nicotine-induced exocrine pancreatic secretion (19–21, 25, 61). Signal transduction involves numerous signals constantly bombarding the cell surface; some may enter the cell and some may bind to cell surface receptors and initiate a flow of information that moves into the cell interior. Stimulation of these receptors activates a group of coupling proteins that regulate a variety of enzymes and ion channels. Target enzymes or ion channels are referred to as effectors because modulations in their activity cause changes in ionic composition or in second messenger levels that ultimately lead to cellular response.

As shown in Figure 1, block 1, the binding of CCK and cholinergic agents to their respective receptors results in the release of inositol phosphate and 1,2-diacylglycerol. In turn, inositol phosphate induces calcium mobilization and activates calmodulin-dependent protein kinases, and 1,2-diacyl glycerol activates and translocates protein kinase C from the cytosolic to the membranous site. Both protein kinase C activation and calcium mobilization are important intermediary steps in pathways of exocrine pancreatic secretion (53, 59).

Data from our investigations suggest that, at least in rats, nicotine induces an inhibition of amylase release as demonstrated by the responsiveness to CCK and carbachol (25). This observation was associated with an increase in the total cellular amylase content. Furthermore, CCK receptor binding capacity measured in isolated membranes showed no difference between control and nicotine-treated acini

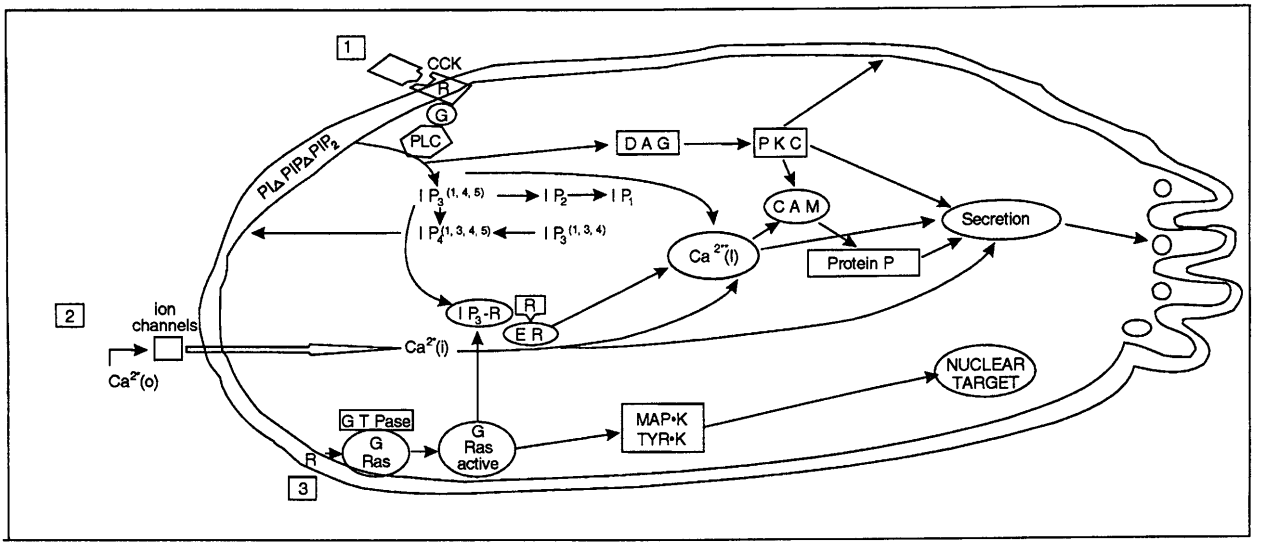

Figure 1. Multiple signal transduction pathways in isolated acinar cell.

(25). These results suggest that postreceptor mechanisms are involved in this altered stimulus-secretion coupling.

Pharmacological Actions of Nicotine

Nicotine is an agonist of the nicotine cholinergic receptor (nAchR) in the central nervous system. It is widely accepted that it exerts pharmacological effects as a result of interactions with these receptors (62–65). There is overwhelming evidence in the literature that the nAChR is the primary site of nicotine action in the CNS (62–65). Several laboratories have demonstrated that prolonged exposure of mice and rats to nicotine and other nicotinic agonists produces a significant increase in the number of agonist binding sites in many brain regions, including cortex, striatum, thalamus, hippocampus, and hypothalamus (64–67). There is also evidence from human postmortem studies indicating that cigarette smokers have increased [^{3}H]-nicotine binding sites in the brain when compared to nonsmokers (68). In contrast to studies in the brain, competitive radioligand binding studies conducted with ^{3}H-nicotine in isolated rat pancreatic acinar cells demonstrated an absence of surface receptors for nicotine (61). However, significant amounts of ^{3}H-nicotine remained bound inside the cytoplasmic compartment of the acinar cell (61). Therefore, the significance of retention of nicotine within the acinar cell and its relationship to pancreatic injury remains to be determined.

It has also been shown that nicotinic receptor activation results in calcium entry through the open nAChR channels (69, 70), increased calcium influx through voltage-dependent calcium channels (71–73), and increased release of intracellular calcium (74, 75) (see Fig. 1, block 2). In bovine adrenal chromaffin cells, it has also been demonstrated that two distinct calcium pools summate within the cell leading to a greater calcium signal (74–76). Recent studies utilizing CCK and carbachol that mobilize intracellular calcium showed an increased accumulation of nicotine in the acinar cell with a correlated decrease in exocrine pancreatic secretion (61). These data strongly suggest that calcium may be the major mediator for alteration in pancreatic secretion induced by nicotine.

Various investigators have also demonstrated that nicotine stimulation of adrenal chromaffin cells lead to an increase in the concentration of inositol trisphosphate (InsP3) (77), InsP4, and InsP5 (78) as well as enhanced translocation of protein kinase C (82) from the cytosol to the membranes. These effects are calcium dependent and can be mimicked by stimulation of the cells with a depolarizing concentration of potassium. Indeed, increases in intracellular calcium promoting cytotoxicity due to nicotine, IP_3 and other agonists in various cellular systems including pancreatic acinar cells, have been reported (80–81).

It is important to examine the relationship between nicotine, intra- and extracellular calcium pools, and intracellular signaling paths. Intracellular signals such as inositol phosphates, protein kinases, diacyl glycerol, and activation of G proteins can lead to calcium release and mobilization. Therefore, future studies may be directed to ascertain whether nicotine affects these pathways (Fig. 1, blocks, 1, 2, 3).

Gene Expression by Nicotine

Regulatory genes can be used to study the various signals for tissue specificity and differential expression of the gene products (82–84). The pattern of pancreatic gene expression has been shown to be extensively modified during pancreatitis (85, 86). The protooncogenes are known to be overexpressed in embryonic tissues (87–89), in the pancreas during carcinogenesis, after induction of growth by mitogens, and during regeneration following pancreatectomy (90). Studies in rats exposed to nicotine *via* inhalation for 21 days showed the enhancement of a mutant *ras* p21 protein expression (91) and activation of the H-*ras* gene in the pancreas acinar cells (92, 93).

Mutations in the *ras* gene alter the normal function of the *ras* gene product, p21 protein, which functions as a signal switch molecule. Altered p21 protein also affects the GTPase activating protein, which mediates the signal transducing effect of p21 (94–96) thereby inactivating the signaling switch (Fig. 1, block 3). Thus, the H-*ras* gene mediated signal transduction pathway might be one of the mechanistic sites by which nicotine induces pancreatic injury. Activation of the *ras* gene is known to trigger the release of inositol phosphates through receptor-mediated G-protein coupling (94, 95), and also stimulate phospholipase C (PLC), which generates "second messengers" such as DAG and IP_3. Consequently, IP_3 stimulates the release of intracellular calcium from endoplasmic reticulum (ER) elevating intracellular Ca^{+2} and predisposes cellular injury (97, 98). Thus, the effects of calcium mobilization *via* H-*ras* and IP_3 in response to nicotine may play an important role in enhancing cell damage in the acinar cells (76, 97, 99).

The proteins encoded by the *ras* gene are essential for the transduction of diverse extracellular signals to intracellular targets (100–103). The *ras* proteins bind guanine nucleotides with high affinity and cycle between an active GTP-bound state and an inactive guanosine diphosphate (GDP)–bound state (104, 105). The *ras* proteins regulate a key point in signal transduction pathways between the mitogenic growth factors and ultimately the nuclear transcription factors that regulate cell division (106–109). It has been shown that nicotine causes oxidative stress to the pancreatic tissue in rats (110) and this appears to be due to the production of free radicals by nicotine. Induction of free radicals could be another factor in the promotion of pancreatic injury by nicotine.

Epidemiological studies demonstrated a significant increase in pancreatic diseases (pancreatitis and carcinoma) in cigarette smokers (4–16). Future studies to explore the relationship of nicotine to gene expression and mutation with cancer development in the pancreas are also warranted.

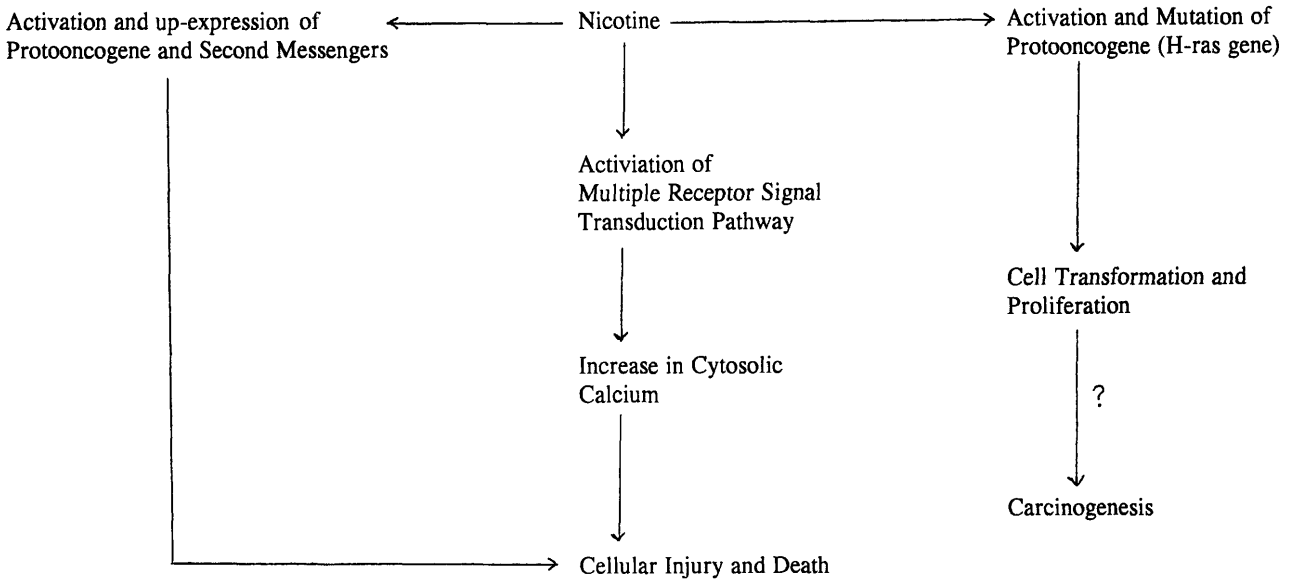

Figure 2. Pathogenetic mechanism of nicotine in the pancreas.

Concluding Remarks

Epidemiological evidence strongly suggests an association between cigarette smoking and pancreatic diseases. The current review focuses on the role of nicotine, a major component in cigarette smoking, on the development of such diseases. Exposure of nicotine to laboratory animals clearly supports the notion that nicotine can induce pancreatic injury. The mechanism is believed to be mediated *via* a signal transduction pathway in the pancreatic acinar cell leading to an increase in intracellular cytosolic calcium that promotes cytotoxicity and eventual cell death. The induction of pancreatic injury by nicotine may also involve activation and expression of a protooncogene (*ras* gene) that can also induce an increase in cytosolic calcium *via* second messenger pathways. Enhancement of pancreatic carcinoma in cigarette smokers, as observed in human populations, may result from activation and mutation of protooncogenes, such as the H-*ras* gene. The pathogenetic mechanism of nicotine in the pancreas is schematically summarized in Figure 2.

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