MINIREVIEW

Pathophysiological Effects of Nicotine on the Pancreas: An Update[1]

PARIMAL CHOWDHURY,[2]* STEWART MACLEOD,† KODETTHOR B. UDUPA,*'‡ AND PHILLIP L. RAYFORD*

*Departments of *Physiology and Biophysics, †Surgery, and ‡Geriatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205*

Epidemiological evidence strongly suggests an association between cigarette smoking and pancreatic diseases. It is well recognized that nicotine, a major component in cigarette smoke, is an addictive agent and, therefore, reinforces smoking behavior. The current review update focuses on the genetics of nicotine dependence and its role on the development of pancreatic diseases. The role of smoking and nicotine in pancreatitis and pancreatic cancer development is also discussed. Exposure of laboratory animals to nicotine clearly supports the notion that nicotine can induce pancreatic injury. The mechanism by which nicotine induces such effects is perhaps mediated via signal transduction pathways in the pancreatic acinar cell, leading to enhanced levels of intracellular calcium release, resulting in cytotoxicity and eventual cell death. The induction of pancreatic injury by nicotine may also involve activation and expression of protooncogene, H-*ras*, which can increase cytosolic calcium via second messenger pathways. Development of pancreatic carcinoma in cigarette smokers as observed in human populations may be the result of activation and mutation of the H-*ras* gene. A possible pathogenetic mechanism of nicotine in the pancreas activating multiple signal transduction pathways is schematically summarized in Figure 1.

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[2] To whom requests for reprints should be addressed at Department of Physiology and Biophysics, Slot #505, University of Arkansas For Medical Sciences, 4301 West Markham Street, Little Rock, Arkansas 72205. E-mail: chowdhuryparimal@uams.edu

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In 1998, we published a mini-review on the pathophysiological effects of nicotine on the pancreas (1). Because the deleterious effects of nicotine as they relate to the use of tobacco are continuously being documented, the purpose of this communication is to provide updated information on nicotine and its effects on the pancreas. In addition, we have added information on the genetics of nicotine dependence and have summarized the possible mechanisms by which nicotine may act in the etiology of pancreatic disorders.

Nicotine, a major component of tobacco and cigarette smoke, is an addictive agent and has been characterized as a drug of abuse by the U.S. Surgeon General (2–4). Approximately 430,000 persons die of causes related to smoking cigarettes and approximately 30% of those deaths are due to some form of cancer (5, 6). In France, pancreatic carcinoma is a major health concern, as it kills more than 6000 people each year (7). Cigarette smoking is the major risk factor in the cause of this disease. These phenomena are a worldwide tragedy because according to research studies, a significant number of patients cease to smoke when advised to do so by a physician (8, 9). 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 (4).

The association of nicotine exposure through cigarette smoking with the increased incidence of pancreatitis and pancreatic cancer has been reported (10–24). A survey on the association between cigarette smoking and pancreatic cancer showed that cigarette smokers had a significantly higher risk (70%) of developing pancreatic cancer in comparison with non-smokers (14–22). When compared with non-smokers, 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 (16). Most of the data linking cigarette smoke/nicotine to pancreatic diseases were gathered in humans. Studies conducted with animals have shown that nicotine or its metabolites could induce pathological and functional changes in the pancreas (25–31). The current review will present an update and discuss our current understandings of the pathophysiology of the exocrine pancreas induced by nicotine. A possible mechanism of action of nicotine on the induction of pancreatic pathology will be discussed. Before we describe the action of nicotine on pancreas, it appears justifiable to review nicotine dependency and the genetics behind this dependency.

The Genetics of Nicotine Dependence

The use of tobacco products, both cigarettes as well as smokeless tobacco, continues to be a major health problem in the United States. An individual's risk of becoming dependent on nicotine may rely on a complex mix of pharmacological, psychological, and socioeconomic factors. However, recent evidence indicates that genetics may be an important factor in the risk of becoming addicted to nicotine. Studies of tobacco product use among twins (32–34), families, and adopted siblings indicate that tobacco use is influenced by heredity (35–37). The influence of an individual's genetic makeup on their risk of nicotine addiction has only recently been explored.

Genes that are involved in nicotine metabolism, and therefore nicotine tolerance, may influence an individual's risk of becoming nicotine dependent. Individuals who are more efficient metabolizers of nicotine may be less likely to have adverse reactions, such as light-headedness and nausea, upon initial exposure and are therefore more likely to continue using tobacco products. Candidate genes involved in tobacco addiction include CYP2A6 and CYP2D6, which are involved in the metabolism of nicotine to cotinine. Pianezza *et al.* (38) found an under-representation of individuals with low-activity alleles for CYP2A6 among a tobacco-dependent group, indicating that individuals with low CYP2A6 activity were less likely to become dependent on nicotine. They also found that smokers with low activity CYP2A6 alleles smoked significantly fewer cigarettes per week, indicating that lower CYP2A6 activity may be related to lower tolerance to nicotine. More recent studies by Oscarson *et al.* (39–41) and Zabetian *et al.* (42) have cast some doubt concerning these results. Using different genotype methods, these groups have found much lower allele frequencies for low activity CYP2A6 among various populations, which may limit the power of studies using genotypes for CYP2A6.

In addition to a direct effect through activation of tobacco-specific nitrosamines such as 4-(*N*-methyl-*N*-nitrosamino)1-(3-pyridyl)-1-butanone (NNK), CYP2D6 may play a role in lifetime tobacco exposure because it also metabolizes nicotine to cotinine and thereby could increase tolerance to nicotine. Although Ayesh *et al.* (43) reported an association between efficient metabolizers of debrisoquin (catalyzed by CYP2D6) and lung cancer risk, subsequent studies have been inconsistent in identifying an association between CYP2D6, smoking, and lung cancer risk (44). This could be due to the complexity of CYP2D6 metabolism of tobacco products. A recent CYP2D6 genotyping study seems to support earlier reports by identifying an association between efficient metabolizers, lung cancer, and moderate smoking exposure (45).

Polymorphic alleles in the D2 dopamine receptor gene affect the availability of dopamine and have been implicated in individual vulnerability to nicotine dependence among tobacco smokers. Nicotine and other drugs such as alcohol and cocaine induce euphoria in users that is thought to be the result of activation of the mesolimbic dopaminergic reward system of the brain (46, 47). Nicotine activates nicotinic receptors that in turn enhance dopamine release in areas of the brain that are thought to be involved in reward (48–50). The involvement of the dopaminergic system in the reinforcement activity of nicotine may be related to the highly addictive properties of the drug. The human dopamine receptor is polymorphic, with two minor alleles termed the *Taq*IA allele (A_1 and A_2) and the *Taq*IB allele (B_1 and B_2). The functional significance of these polymorphic alleles has been determined using labeled D2 dopamine receptor ligands *in vitro* (51) and *in vivo* (52). These experiments show reduced numbers of D2 dopamine receptors in the brains of individuals who were homozygous or heterozygous for the A_1 allele (A_1A_1 or A_1A_2) compared with those who were homozygous for the more common allele (A_2A_2) at this locus. Because the dopamine receptor D2 (DRD2) is an integral part of the dopaminergic reward system, subjects with reduced numbers of dopamine receptors may compensate for this deficiency by using nicotine to increase brain dopamine levels.

Spitz *et al.* (53), in a case control study of lung cancer patients, found that the B_1B_2 genotype was more common in chronic smokers compared with non-smokers, whether they were cases or controls. Smokers with the least common A_1 or B_1 alleles tended to be younger when they started smoking and attempted to quit smoking fewer times compared with smokers with the more common DRD2 alleles. Noble *et al.* (37) determined that allele frequencies for the A_1 allele were higher in current and former smokers (45.6% and 40.0%, respectively) compared with non-smokers (28.0%). In a similar study, Comings *et al.* (54) found that smokers who were unsuccessful in quitting had an allele frequency of 48.7% for the DRD2 A_1 allele compared with 18.2% for non-smokers. In addition, light smokers had a 37.5% allele frequency for A_1 versus 52.2% for heavy smokers. Taken together, these data suggest that the polymorphic alleles of the DRD2 may be predictive markers for individuals who are at risk of becoming addicted to nicotine.

A functional polymorphism in the promoter region of the serotonin transporter gene (5-hydroxytryptamine transporter or 5-HTT) consists of a 44-bp deletion/insertion that

corresponds to short (s) and long (l) versions of the promoter. The short promoter variant reduces the transcriptional activity of the gene and results in decreased 5-HTT expression (55). Evidence for the function of this polymorphism includes transfection of lymphoblastoid cells with reporter vectors containing the long and short forms of the 5-HTT promoter polymorphism. It was found that the basal and induced activity of the l form was twice that of the s form. In addition, the expression of the native 5-HTT gene in lymphoblastoid cell lines from subjects with different 5-HTT genotypes was found to vary. Cells from l form homozygotes produced 1.4 to 1.7 times as much 5-HTT mRNA compared with homozygotes with the s form of the gene. In addition, [^{3}H]5-HT uptake in cells that were homozygous for the l form of 5-HTT was 1.9–2.2 times that of cells with either the heterozygous or the homozygous s form of the gene. Taken together, these data demonstrate that the s form of the 5-HTT promoter polymorphism is responsible for lower production of 5-HTT and would be expected to produce lower levels of serotonin reuptake in individuals with this form of the gene.

Deficiency in serotonin reuptake may increase the risk of impulsive/aggressive behavior as well as the risk of depression. Neuroticism is a set of personality traits that include anxiety, depression, impulsiveness, and vulnerability factors that have been implicated in the risk of becoming a smoker as well as becoming dependent on nicotine. These traits have also been associated with difficulty in quitting smoking. There is evidence that the 5-HTT promoter polymorphism is related to smoking behavior and to nicotine dependence. Hu *et al.* (56) determined the 5-HTT promoter polymorphism genotype for 759 current, former, and lifelong smokers and found a relationship between smoking behavior, neuroticism, and 5-HTT genotype. Lerman *et al.* (57) also found that smokers who were heterozygous or homozygous for the s allele for 5-HTT were more likely to be dependent on nicotine compared with those who were homozygous for the l allele.

The action of dopamine is terminated by the catabolic action of the enzymes catecholamine-*O*-methyltransferase (COMT) and monoamine oxidase (MAO). Both of these enzymes are polymorphic, and allelic variants of these enzymes that confer different activity on their respective enzymes may contribute to individual differences in susceptibility to substance abuse, including nicotine. A common single nucleotide polymorphism in the COMT gene results in an amino acid change of valine to methionine at residue 108 or 158 in soluble and membrane-bound COMT, respectively. The variants exhibit a 3- to 4-fold variation in COMT activity, with the methionine containing variant having low activity. Higher activity of COMT and/ or MAO could be responsible for lower dopamine levels, and individuals with lower dopamine levels may compensate by using nicotine products.

Future studies designed to explore interindividual differences in nicotine and dopamine metabolism will be useful in identifying individuals who are at increased risk of becoming dependent on nicotine. The results of these studies will also facilitate the development of smoking cessation strategies that are targeted to individual differences in nicotine and dopamine metabolism. Before we discuss the effect of nicotine on the pancreas, a brief description of the anatomy of the pancreas is given below.

Anatomy of the Pancreas

The human pancreas weights about 80 g and has two major sections. The endocrine section makes up approximately 2% of the gland, whereas the exocrine section is about 85% of its total mass (58). Nerves, blood vessels, and other tissues make up the remaining portion of the pancreas. There are four major regions of the pancreas: head, tail, body, and neck; these are named according to their anatomical position in the gland. The fine structures of the pancreas are designated as: (a) acinar units, these cells contain zymogen granules that store pancreatic enzymes; (b) ductle units, these cells store water and bicarbonate; (c) basal lamina, is that portion of the pancreas that interfaces between connective tissue and epithelial cells and is composed of laminin, collagen and fibronectin; and, (d) islet cells. The endocrine pancreas has at least four types of cells: A cells secrete glucagon, B cells secrete insulin, D cells secrete somatostatin, and PP cells secrete pancreatic polypeptide (59). Each of these hormones is involved in the regulation of metabolism. The exocrine pancreas is affected mostly by smoking and probably by alcohol abuse.

Pancreatitis And Pancreatic Cancer: Association with Smoking. An estimated 3 million deaths occur worldwide due to tobacco use (60, 61), and it was shown that the number of deaths from pancreatic cancer was 2.5 times higher in women than in men (60). Besides the demographic factors, cigarette smoking has been suggested as the single most important factor for the development of these diseases (62–65). Several studies showed that patients with chronic pancreatitis had an increased risk of developing pancreatic cancer (66–68). An evaluation of 37,450 patients with unspecified, acute, recurrent, and chronic pancreatitis showed an increased risk of pancreatic cancer development in all sub-cohorts (69). Very few animal models of pancreatitis and pancreatic cancer are available to study the etiology of these diseases; however, studies reported in the hamster model showed recurrent pancreatitis resulting in large number and size of pancreatic tumors (70). More recently, mouse models of exocrine pancreatic cancer have been described (71). These models attempted to address the interaction between a genetic change and the tissue, organ, and whole-animal homeostatic mechanisms that tend to restrict unregulated tissue growth. Evidence from these models suggests that ductal neoplasia may be an important component of exocrine pancreatic cancer progression (71).

Metabolism of Nicotine and Its Effects on the Structural and Functional Changes of the Exocrine Pancreas. Cotinine and nornicotine are natural metabolites of nicotine. Many of the pharmacological effects of

tobacco smoking are due to nicotine (72). Nitrosoamine derivatives of nicotine and other metabolites have been reported to be carcinogenic (73, 74). About 80%–90% of the dose of nicotine consumed can be accounted in human urinary metabolites. It has been reported that nicotine is biotransformed to highly reactive chemicals that covalently modify proteins and DNA (75–77). In rodents, hepatic nicotine metabolism is found to involve cytochrome p450s that catalyze the first step of this pathway (78, 79, 81). Major defective CYP2A6 alleles were recently found in cigarette smokers (80, 40–42).

It has been demonstrated that pancreatitis could be induced in mice fed on a caerulein- and choline-deficient ethionine (CDE) supplemented diet (82–87). 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 (87). The vacuoles were found to contain digestive and lysosomal enzymes (88–90), and upon activation, they promote degenerative changes in the pancreas (90, 91). Exposure of animals to nicotine has been shown to induce morphological changes in the pancreas similar to those induced by caerulein and CDE diets (25, 26, 29–31). However, it is 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 obscure. Tobacco smoking, diabetes mellitus, cholelithiasis, and pancreatitis increase the risk of pancreatic cancer (92). Hedberg and colleagues (93) found that a disturbed regulation of pancreatic enzyme secretion, or a regulatory dysfunction of the pancreatic gland, could contribute to the development of pancreatic carcinoma in patients after partial gastrectomy. Their studies suggest that the hormone CCK, which was shown to induce experimental neoplasia, is not involved in this phenomenon. In 30 patients with chronic pancreatitis, it has been reported that postprandial levels of CCK were increased above those found in normal patients (94). There was no difference in basal levels of CCK and in basal and postprandial levels of somatostatin between normal and diseased patients.

We have shown that rats given nicotine in their water ate approximately 10% less food than controls given only water (95). In a separated group of rats given 10% less food than other rats that were allowed to eat food *ad libitum*, rats on less food in each study showed loss of body weight and impaired pancreatic enzyme secretion (96). In a study with humans, Winter and colleagues (97) showed that severe undernourishment resulted in primary gastric and pancreatic secretory dysfunction. In these studies, pancreatic injury was not assessed; however, the studies do suggest that nicotine, by reducing food intake, may play a role in panereatic injury in humans.

Effects of Nicotine on Gastrointestinal Function. Gastrointestinal secretions in man are affected by cigarette smoking (99). Evidence suggests that nicotine has a direct effect on pancreatic secretions (26–30, 99–102). It has been shown that when rabbits were exposed to nicotine, there was a significant decrease in secretion of duodenal bicarbonate (102). A decreased responsiveness to secretagogues in the pancreas was also found in rats exposed to nicotine (25, 26, 30, 31). Exposure of isolated pancreatic acini to nicotine *in vitro* enhanced secretion of hydrolases and newly synthesized proteins (29). These studies suggest that nicotine and its metabolites have a definitive effect on exocrine pancreatic secretions.

In a study with 207 patients, Heilkius and colleagues (103) concluded that levels of pancreatic enzymes are elevated in a significant proportion of patients with inflammatory bowel disease and with more extensive and active disease. In addition, sclerosing cholangitis also seemed to be associated with increases in pancreatic enzymes. In these patients, urinary amylase levels were higher in smokers than in non-smokers and ex-smokers. Brown (98), in a study with 14 patients, has shown that the smoking of only one cigarette can result in decreased volume and bicarbonate output by the pancreas. He concludes that this effect of nicotine may play a role in the formation of duodenal ulcers in humans.

The studies reported in humans and in our animal studies are consistent in the fact that in both instances, the total content of pancreatic enzymes are elevated in the pancreas with either smoking or nicotine. However, responsiveness of pancreatic enzyme secretion by CCK from isolated acinar cells from animals exposed to nicotine was significantly decreased. The reduced secretion and increase in enzyme content in the pancreas may be a trigger for induced pathogenesis. The dose-response and time course effect of nicotine-induced pathology (e.g., edematous, vacuolar, and pyknotic changes, as well as alterations in mitochondria and other organelles) need to be further examined and characterized.

Chronic cigarette smoking has been directly linked to pulmonary emphysema with correlated reduction in endogenous antiproteases (104). It has been shown that changes in the basal serum levels of the gastrointestinal hormones CCK (105–108), or serum enzymes such as amylase and lipase (109–112), were associated with pancreatic injury (110–113). It is of interest to note that when subjected to a single injection of secretin, there were a significantly higher serum concentrations of pancreatic digestive enzymes in smokers than in non-smoking controls (114), suggesting some form of pancreatic injury occurred in smokers.

Possible Mechanism of Action of Nicotine on the Exocrine Pancreas. Gastrointestinal hormones such as CCK, carbachol, and secretin are ligands for pancreatic receptors and act through specific receptor pathways, mediating complex signal transduction events, resulting in exo-

cytosis of pancreatic enzymes from zymogen granules of the acinar cells (107–109, 115).

A pancreatic acinar cell model as described earlier by many investigators (109, 115–120) demonstrates the mechanism of underlying pathologic changes induced by nicotine (Fig. 1). 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 that 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 (109, 115, 117, 121). Two major classes of receptors were identified in acinar cells based on their response to different agonists: those coupled to mobilization of cellular calcium (CCK, bombesin, and carbachol) and those coupled to activation of adenylate cyclase (secretin and vasoactive intestinal peptide) (115, 121, 122).

A schematic diagram describing the multiple signal transduction pathways in an acinar cell 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 and retention of enzymes (25–27, 31, 122). As shown in Figure 1 (pathway 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. 1, 2-Diacyl glycerol activates and translocates protein kinase C from the cytosolic to membranous site. Both protein kinase C activation and calcium mobilization are important intermediary steps for pathways of exocrine pancreatic secretion (115, 121).

Data from our investigations suggested that, at least in rats, nicotine induced an inhibition of amylase release, as demonstrated by their responsiveness to CCK and carbachol (31). 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 (31). These results suggest that postreceptor mechanisms are involved in this altered stimulus-secretion coupling. (A second possible pathway as shown in Figure 1, pathway 2.)

Nicotine is an agonist of the nicotine cholinergic receptor (nAchR) in the central nervous system (CNS) (123). It is widely accepted that it exerts pharmacological effects as a result of interactions with these receptors (123–126). Significant evidence exist in the literature that suggests that nAChRs is the primary site of nicotine action in the CNS (123–126). 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 (125–128). There is also evidence from human *post mortem* studies indicating that cigarette smokers have increased [^{3}H]nicotine binding sites in the brain when compared with non-smokers (129).

It has also been shown that nicotinic receptor activation would result in calcium entry through the open nAChR channels (130, 131), increased calcium influx through voltage-dependent calcium channels (132–134), and increased release of intracellular calcium (135, 136). In bovine adrenal chromaffin cells, it has also been demonstrated that there were two distinct calcium pools that summate within the cell leading to a greater calcium signal (135–137). Com-

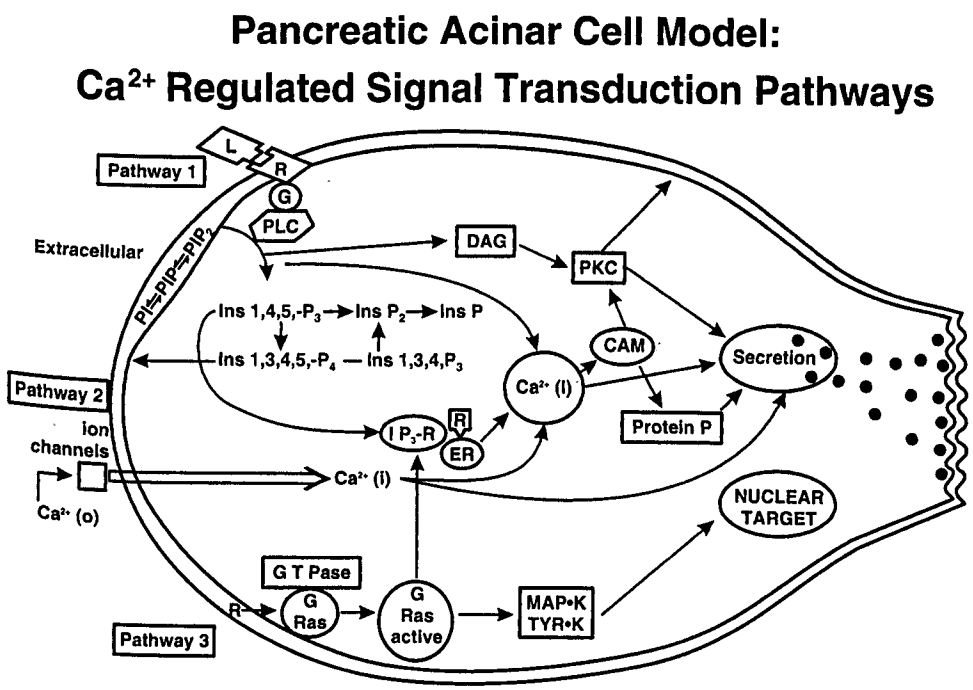

Figure 1. Pancreatic acinar cell model showing induction of multiple Ca^{2+} regulated signal transduction pathways (Printed with permission from Lippincott Williams & Wilkins. Eur J Gastroenterol Hepatol 12(8): 869–877, 2000).

petitive radioligand binding studies conducted with ^{3}H-nicotine in isolated rat pancreatic acinar cells in our laboratory showed no or little binding of nicotine to surface receptors (122). A significant amount of ^{3}H-nicotine remained bound inside the cytoplasmic compartment of the acinar cell (122). Further studies show that CCK and carbachol, which mobilize intracellular calcium, facilitated the increased accumulation of nicotine in isolated pancreatic acinar cells (123), suggesting the involvement of intra- and extracellular calcium as major mediators of nicotine entry into the acinar cells and inducing altered exocrine pancreatic secretion.

Various investigators have also demonstrated that nicotine stimulation of adrenal chromaffin cells led to an increase in the concentration of inositol trisphosphate (InsP3) (138), InsP4, and InsP5 (139), as well as enhanced translocation of protein kinase C (140) 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 (141, 142).

It appears important to examine the relationship between nicotine, intra- and extracellular calcium pools, and intracellular signaling paths. Data from our laboratory suggest that the entry of nicotine into the acinar cell is perhaps regulated by Ca^{2+} receptor pathways (122). 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 (see pathways 1, 2, and 3 in Fig. 1). (A third possible pathway of altered exocrine pancreatic pathology may be due to overexpression of oncogenes by nicotine as shown in pathway 3, Fig. 1.)

Regulatory genes can be used to study the various signals for tissue specificity and differential expression of the gene products (143–145). The pattern of pancreatic gene expression is extensively modified during pancreatitis (146, 147). The proto-oncogenes are overexpressed in embryonic tissues (148–150), in the pancreas during carcinogenesis, after induction of growth by mitogens, and during regeneration following pancreatectomy (151). Studies in rats exposed to nicotine via inhalation for 21 days showed the enhancement of expression of a mutant ras p21 protein and activation of the H-*ras* gene in the acinar cells of the pancreas (152).

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 (153–155), thereby inactivating the signaling switch. 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 product triggers the release of inositol phosphates through receptor-mediated G-protein coupling (153, 154), and also stimulates phospholipase C (PLC) generating "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 (156, 157). 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 (138, 156, 158).

The proteins encoded by the *ras* gene are essential for the transduction of diverse extracellular signals to intracellular targets (159–162). 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 (163, 164). 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 (165–168).

Thus, the activation of these multiple signal transduction pathways due to nicotine exposure results in high levels of intracellular calcium release and may be responsible for cell cytotoxicity and cell injury. Future studies to explore the relationship of nicotine on gene expression and mutation and with cancer development in the pancreas are also warranted.

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