



Review

Nicotine, its metabolism and an overview of its biological effects

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Abstract

Nicotine is a naturally occurring alkaloid found in many plants. The principal sources of nicotine exposure is through the use of tobacco, nicotine containing gum and nicotine replacement therapies. Nicotine is an amine composed of pyridine and pyrrolidine rings. It has been shown that nicotine crosses biological membranes and the blood brain barrier easily. The absorbed nicotine is extensively metabolized in the liver to form a wide variety of metabolites including nicotine *N'*-oxide and cotinine *N'*-oxide. These are the products of mixed function oxidase system. Nicotine is also converted to some biologically important compounds during harvesting. Among these are the nitrosamines specific to tobacco. Nicotine has been shown to affect a wide variety of biological functions ranging from gene expression, regulation of hormone secretion and enzyme activities. The objective of this study was to overview the biological effects and metabolism of nicotine.

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Keywords: Nicotine; Metabolism of nicotine; Effects of nicotine**Contents**

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1. Introduction

Nicotine is found in a wide variety of plants (Doolittle et al., 1995). However, the principal source of nicotine exposure is through the use of tobacco and nicotine replacement therapies such as transdermal nicotine patches and nicotine containing gum (Heisheman et al., 1994). Nicotine is an amine composed of pyridine and pyrrolidine rings (Schevelbein, 1982). It has been shown that nicotine can cross the biological membranes including the blood brain barrier. Once absorbed, nicotine is extensively metabolized by the liver to a number of major and minor metabolites. (Snyder et al., 1993; Cashman et al., 1992; Neurath, 1994; Crooks and Godin, 1988; Godin and Crooks, 1986; Booth and Boyland, 1971; Kyerematen et al., 1990). Nicotine is also converted to a number of biologically important compounds during harvesting and fermentation. The most pronounced are tobacco specific nitrosamines (Brunneman et al., 1996). The actions of nicotine have been extensively investigated in human, in animal, and in a variety of cell systems. The predominant effects of nicotine in the whole intact animal or human consist of an increase in pulse rate, blood pressure, and an increase in plasma free fatty acids, a mobilization of blood sugar, and an increase in the level of catecholamines in the blood (Benowitz, 1988; Dani and Heinemann, 1996; Waldum et al., 1996; Ashakumary and Vijayammal, 1991). In addition, nicotine has also been found to disturb the antioxidant defense mechanisms in rats fed a high fat diet. At the cellular level, stimulation of nicotinic receptors leads to increased synthesis and exocytic release of several hormones such as nor epinephrine and epinephrine (Yoshida et al., 1980; Goodman, 1974). In addition to the release of these hormones, chronic nicotine treatment has also been shown to activate tyrosine hydroxylase, the first and the rate limiting enzyme in catecholamine biosynthesis (Hiremagular et al., 1993; Fluharty et al., 1985). Nicotinic receptor stimulation has also been shown to induce the transcription factors c-fos and c-jun and to stabilize the intracellular levels of transforming growth factors (Slotkin et al., 1997; Rakowicz et al., 1994). The other effects of nicotine at the cellular level are increased expression of heat shock proteins, induction of sister chromatide exchange and chromosome aberration, inhibition of cell proliferation, and suppression of apoptosis (Hahn et al., 1991; Doolittle et al., 1995; Trivedi et al., 1990, 1993; Maneckjee and Minna, 1994; Yamashita and Nakamura, 1996; Aoshiba et al., 1996; Tipton and Dabbous, 1995).

1.1. Chemical properties of nicotine

Nicotine is a naturally occurring alkaloid found primarily in the members of the solanaceous plant family such as potato, tomato, green pepper, and tobacco (Doolittle et al., 1995). Nicotine was first isolated and determined to be the major constituent of tobacco in 1828 (Schevelbein, 1982).

It has an active center and occurs as stereo isomers (Borlow and Hamilton, 1965). The structure of nicotine [1-methyl-2-(3-pyridyl-pyrrolidine), $C_{10}H_{14}N_2$] was proposed by researchers in 1892 and confirmed in 1895 by synthesis (Pictet and Crepieux, 1895). Pure nicotine is a clear liquid with a characteristic odour whereas it turns brown on exposure to air (Schevelbein, 1962). It can mix with an equal amount of water. However, it partitions preferentially into organic solvents. Thus, it can easily be extracted from aqueous solutions by solvent extraction. It is a strong base and has a boiling point of 274.5 °C at 760 Torr (Schevelbein, 1962).

1.2. Absorption of nicotine

Nicotine absorption can occur through the oral cavity, skin, lung, urinary bladder, and gastrointestinal tract (Schevelbein et al., 1973). The rate of nicotine absorption through the biological membranes is a pH dependent process (Schevelbein et al., 1973). The presence of both a pyrrolidine and a pyridine nitrogen means that nicotine is dibasic with pKa of 7.84 and 3.04 at 25 °C. The proportion of uncharged nicotine increases as the pH of the solution containing nicotine increases whereas the proportion of charged nicotine increases as the pH of the solution containing nicotine decreases. Uncharged organic bases are lipophilic (fat soluble) whereas charged organic bases are hydrophilic (water soluble). The rate of nicotine absorption through biological membranes thus increases as the pH of the aqueous solution increases whereas nicotine absorption decreases as the pH decreases. The absorption of nicotine through the oral mucosa has been shown to be the principal route of absorption for smokers who do not inhale and for smokeless tobacco users. The pH of cigarette tobacco is about 5.5 and nicotine at this pH is largely positively charged. Thus, nicotine is little absorbed via the buccal mucosa. The principal route of nicotine absorption in smokers who inhale is through the alveoli of the lung (Armitage, 1974). The pH of the aqueous fraction of cigar smoke is generally around 8.5 (Russel et al., 1980) and the pH of the alveoli is about 7.4 and at this pH about 31% of the nicotine is uncharged and therefore passes easily across the cell membrane into the circulation. Absorption through the alveoli is also dependent on the nicotine concentration in the smoke. It has been shown that the plasma nicotine level in non-inhaling smokers is around 2.5–8.0 ng/ml, whereas the plasma nicotine levels in inhaling smokers reach 30–40 ng/ml nicotine. These observations demonstrated that absorption of nicotine through the buccal mucosa is poor and absorption through the lung is rapid. Nicotine is also absorbed through the skin (Schevelbein et al., 1972). Absorption of nicotine through the skin is important during tobacco harvesting and during nicotine replacement therapies. The reabsorption of the extracted nicotine occurs through the urinary bladder (Barzelleca, 1963). The absorption of nicotine from the urinary bladder seems to

be dependent on pH of the urine. The movement of nicotine through the mucosa of the bladder is increased when the alkaloid is non-ionized and pH is between 8.0 and 9.0. Nicotine cannot be reabsorbed if the pH of the urine is below 6.0. The nicotine absorption through the gastrointestinal tract is poor because of the acidic nature of the stomach juice (Travel, 1940).

1.3. Metabolism of nicotine

Studies on nicotine metabolism were advanced by the use of several methods involving enzyme purification, purification of specific antibodies, immunochemical and biochemical methods, and high pressure liquid chromatography (HPLC) (Nakayama, 1988). Studies on cytochrome P450 and flavin adenine dinucleotide (FAD) containing monooxygenases produced remarkable progress on analysis of nicotine metabolism (Nakayama, 1988). The biochemical techniques suggested the participation of cytochrome P-450 and FAD-containing monooxygenases in microsomal nicotine metabolism (Peyton et al., 1988). Metabolism of nicotine in living organisms is complicated. Pathways of nicotine metabolism could be discussed as phases I and II metabolism of nicotine. The phase I metabolism involves the microsomal oxidation of nicotine and falls into four groups. The phase II metabolism involves N- and O-glucuronidation of nicotine and its metabolites.

1.3.1. Phase I metabolism

1.3.1.1. C-oxidation and conversion of 5'-hydroxy-nicotine into 3-pyridylacetic acid. In most of the people nicotine is 70–80% metabolized to cotinine by C-oxidation. Cotinine was identified as a urinary metabolite in man, rabbit, mouse, and rat (McKennis et al., 1957). The proposed mechanism of conversion of nicotine to cotinine involves hydroxylation of nicotine by microsomal enzymes and conversion to the corresponding aldehyde and production of cotinine by a cytosolic enzyme (Hucker et al., 1960). It was reported that nicotine iminium ion was first formed by loss of water from 5'-hydroxynicotine and converted to cotinine (Murphy, 1973). γ -(3-pyridyl)- γ -oxo-N-methylbutyramide is a metabolite which is isolated following administration of cotinine (McKennis et al., 1960). It was then detected in urine of humans. This amide was also isolated following administration of radiolabelled nicotine to dogs. 3-Pyridyl acetic acid was isolated and identified as a metabolite following administration of (–)-cotinine (McKennis et al., 1961). Furthermore, other intermediates, demethyl cotinine and δ -(3-pyridyl)- γ -oxobutyric acid were reported to be mainly converted to 3-pyridylacetic acid following administration of these metabolites to animals (McKennis et al., 1964).

The enzymes involved in the C-oxidation of nicotine is now being mostly identified. The most important enzyme in the C-oxidation of nicotine leading to the cotinine formation is CYP2A6, formerly known as coumarin 7-hydroxylase (Nakajima et al., 1996; Messina et al., 1997). It has been

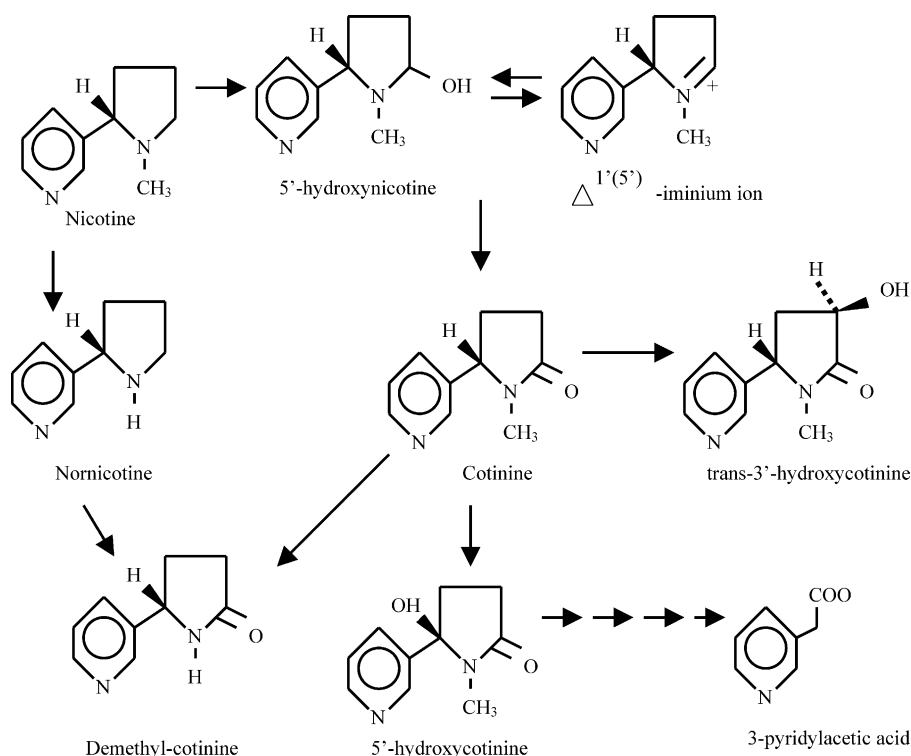


Fig. 1. C-oxidation.

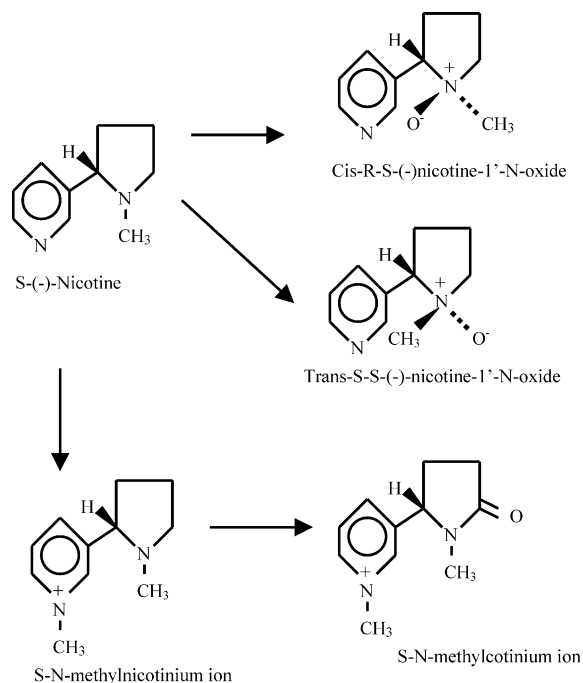


Fig. 2. N-oxidation and methylation.

shown that there is genetic polymorphism in human CYP2A6. The CYP2A6^{*9} allele have been shown to result in decreased expression level and enzymatic activity of CYP2A6 (Yoshida et al., 2003). Researchers also studied the impairment of nicotine metabolism by CYP2A6^{*4}, CYP2A6^{*7} and CYP2A6^{*10} (Yoshida et al., 2002). It is suggested that the efficiency of the conversion of nicotine to cotinine and the variations in the nicotine metabolism is affected by the relative roles of the polymorphic enzymes. Thus, genetic polymorphism in nicotine metabolism is suggested to be an important factor in individual's smoking behavior (Nakajima et al., 2001). However, contradictory results have been published that does not support this hypothesis (Tricker, 2003). Genetic polymorphism of CYP2A6 gene and tobacco induced lung cancer risk was also investigated. It was reported that subjects with ^{*4}/^{*4} genotypes have low risk for lung cancer whereas ^{*1}/^{*1} genotypes have higher risk for lung cancer (Ariyoshi et al., 2002). Same researchers suggested that complete lack of CYP2A6 affects the smoking behavior. Because smokers with the ^{*4}/^{*4} genotype displayed a significant reduction of daily cigarette consumption. However, this suggestion is again contradictory to other researcher's results which suggests that CYP2A6 gene deletion is associated with decreased lung cancer but is not associated with reduced tendency to smoke (Tan et al., 2001).

In some cases, the deficiency of C-oxidation of nicotine has also been reported. In one case study, it was reported that case subject converted only 9% of nicotine to cotinine

compared to the control prolonging the half life of nicotine in the organism (Benowitz et al., 1995).

1.3.1.2. Formation of nornicotine, demethyl cotinine, trans-3-hydroxy-cotinine and δ -(3-pyridyl)- γ -methylaminobutyric acid. Demethyl cotinine, nornicotine, and 3-hydroxycotinine were isolated and determined as urinary metabolites following administration of (-)-nicotine and (-)-cotinine to humans and animals (Bowman and McKennis, 1962). 3-Hydroxycotinine has been reported to be formed from ¹⁴C-(-)-nicotine (Hansson et al., 1964). It was shown that nicotine-methyl-¹⁴C was converted to 3-hydroxycotinine, cotinine and δ -(3-pyridyl)- γ -oxo-N-methylbutyramide, whereas, ¹⁴C-cotinine was converted to 3-hydroxycotinine, demethylcotinine and γ -(3-pyridyl)- γ -oxo-N-methylbutyramide (Stalhandsko, 1970). It has also been shown that 5-hydroxycotinine formed from (-)-nicotine leads to a product which consists of a tautomeric mixture of the hydroxylactam and ketoamide (Nyugen et al., 1981).

1.3.1.3. N-oxidation. Nicotine-1'-N-oxide was first determined in a reaction mixture of rabbit liver extract (Papadopoulos, 1964a). Nicotine-N-1-oxide formation is catalyzed by hepatic flavin-containing monooxygenase 3 (FMO3). N-oxidation of cotinine also takes place. However, the enzyme involved in N-oxidation of cotinine has not yet been identified. Diastereospecific kinetic studies of nicotine oxidation by porcine liver FMO showed a stereoselectivity in the formation of diastereomeric N'-oxides (Nakajima et al., 1998). It was shown that (S)-(-)-nicotine exhibited no stereoselectivity in the formation of *cis*-1'R, 2'S- and *trans*-1'S, 2'S-products whereas with R-(+)-nicotine, only the *trans*-1'R, 2'R-N'-oxide was formed (Damani et al., 1988). These results indicated that (R)-(+)-nicotine binding might be sterically inhibited when N'-methyl and pyridyl groups are in a *cis*-orientation. Except in liver extracts, nicotine-1'-N-oxide has also been detected as a urinary metabolite in animals and in humans (Papadopoulos, 1964b).

1.3.1.4. N-methylation. The other pathways of nicotine metabolism involves N-methylation and N-demethylation of nicotine (Gorrod and Schepers, 1999). It has been shown that following administration of (-)-nicotine and (-)-cotinine to animals isomethylnicotinium and cotinine methonium ions are formed as urinary metabolites (McKennis et al., 1963). It has been shown that nicotine N-methylation in guinea pigs is a stereo specific reaction involving only the (+)-nicotine (Cundy et al., 1984). Methylation of nicotine enantiomers is also studied in human liver cytosol. A similar substrate stereoselectivity was observed in human liver cytosol (Crooks and Godin, 1988).

1.3.2. Phase II metabolism

1.3.2.1. N- and O-glucuronidation. Following the characterization of phase I metabolism of nicotine by P450 (CYP) researchers studied the phase II glucuronidation of nicotine

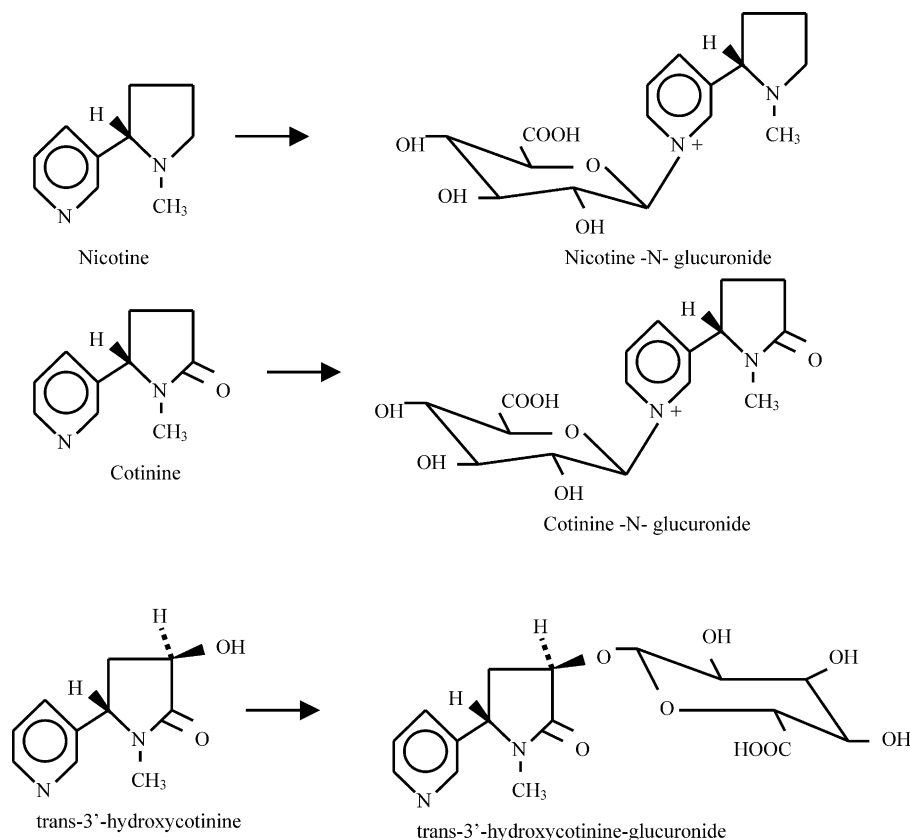


Fig. 3. N- and O-glucuronidation.

and its metabolites. UDP-glucuronosyltransferase (UGT) enzymes are involved in conjugation with glucuronide. These enzymes are known to produce more water-soluble compounds compared to the parent compounds. Conjugates of nicotine and its metabolites with glucuronide, nicotine and cotinine N-glucuronides, are excreted in the urine (Byrd et al., 1992; Caldwell et al., 1992; Tsai and Gorrod, 1999). Recently, nicotine and cotinine N-glucuronidations in human liver microsomes were studied and characterized (Nakajima et al., 2002). This study showed that nicotine N-glucuronidation is biphasic and involves more than one enzyme and cotinine N-glucuronidation is monophasic. Enzyme inhibition approach in this study also demonstrated that nicotine and cotinine glucuronidation involves UGT1A1, UGT1A9 and UGT1A4. Another CYP metabolite of nicotine, *trans*-3'-hydroxycotinine, is conjugated by O-glucuronidation (Tricker, 2003; Benowitz and Jacob, 2000).

1.4. Nicotine excretion

It has been demonstrated that nicotine could be excreted through urine, feces, bile, saliva, gastric juice, sweat, and breast fluid (Perlman et al., 1942; Hansson and

Schmitterloew, 1962; Fishman, 1963; Turner, 1969; Balabanova et al., 1992; Seaton et al., 1993). When ^{14}C -nicotine is given to an animal, it has been shown that around 55% of the radioactivity is excreted in the urine. However, only 1% of the radioactivity was observed in the form of unchanged nicotine. This result demonstrates that nicotine is excreted following extensive metabolism. The urinary excretion of nicotine and its metabolites has been shown to be affected by ascorbic acid. It was reported that ascorbic acid increases the urinary excretion of cotinine and nicotine (Dawson et al., 1999). Nicotine and cotinine is also determined in the urine of infants who have mothers who smoke indicating that exposure of mothers to tobacco smoke affect the infants (Luck and Nau, 1985). In another study, the hypothesis that the rate of renal excretion of nicotine influences the nicotine intake during smoking was investigated. Researchers reported that the daily nicotine intake was 18% higher in persons with increased nicotine excretion and concluded that the rate of elimination of nicotine affects the rate of consumption (Benowitz and Jacob, 1985). As discussed in Section 1.2 in detail, the rate of nicotine excretion is also influenced by the pH of the urine. When the pH of the urine is made alkaline the proportion of uncharged nicotine increases and reabsorption of nicotine occurs and as

Table 1
The effects of nicotine in whole organisms and in cells

| The effects in the whole organism | The effects at the cellular level |
|---|---|
| Increased heart rate | Increased synthesis and release of hormones |
| Cardiac contractility | Activation of tyrosine hydroxylase enzyme |
| Increased blood pressure | Activation of several transcription factors |
| Decreased skin temperature | Induction of heat shock proteins |
| Mobilization of blood sugar | Induction of oxidative stress |
| Increase in free fatty acids in the blood | Effects on apoptosis |
| Increased catecholamine levels in the blood | Induction of chromosome aberrations |
| Arousal or relaxation | Induction of sister chromatide exchange |

a result, less nicotine is excreted (Becket et al., 1965). However, when the urine is made acidic the proportion of charged nicotine increases thus less nicotine is reabsorbed and more nicotine is excreted.

1.5. The biological effects of nicotine

The effects of nicotine have been extensively investigated in animals and in a variety of cell systems. Nicotine has been shown to have a wide variety of effects on multicellular organisms and single cells. These effects are summarized in Table 1.

1.5.1. The effects of nicotine on oxidative stress

Nicotine, the major component of the cigarette smoke, plays an important role in the development of cardiovascular disease and lung cancer (Anna et al., 1987; Heusch and Maneckjee, 1998). Lipid peroxidation and generation of free radicals are the processes associated with the pathogenesis of atherosclerosis (Morel et al., 1963). It has been shown that the level of lipid peroxidation increases in smokers (Pre et al., 1989). In nicotine administered rats, the concentrations of free fatty acids and the level of malondialdehyde, hydroperoxides, and conjugated dienes also increased. The increased free fatty acids in tissues of nicotine-treated rats may serve as substrate for lipid peroxidation. It has also been shown that nicotine administration results in a decrease in the activities of free radical scavenging enzymes superoxide dismutase, catalase, and glutathione reductase (Ashakumary and Vijayammal, 1996). A decrease in activities of these free radical scavenging enzymes results in increased generation of superoxide anion and hydrogen peroxide which in turn results in generation of hydroxyl free radicals. Generation of hydroxyl free radicals has been shown to participate in many toxic reactions (Halliwell

and Guttridge, 1984, 1988). Increased production of superoxide and hydrogen peroxide may cause deleterious injury to alveolar macrophages thereby causing release of proteolytic enzymes (Millane et al., 1984; Romson et al., 1983). The products of lipid peroxidation, which are increased following nicotine administration, may cause irreversible damage to the membrane structure of the cells. Consistent with this, it was shown that in vitro nicotine administration resulted in increased leakage of lactate dehydrogenase enzyme whose leakage into the media indicates the presence of membrane damage. In similar studies, it was demonstrated that nicotine potentiates superoxide generation by human neutrophils and by polymorphonuclear leukocytes (Gillespie et al., 1987). Generation of cytotoxic neutrophil derived free radicals have been shown to be implicated in the pathogenesis of cardiovascular, pulmonary and neoplastic disorders. In the same study, it was shown that the alkaloid nicotine fails to induce neutrophil oxidative metabolism alone. However, nicotine potentiates the superoxide release induced by phorbolmyristate acetate. Potentiation was not inhibited by nicotinic receptor antagonists indicating that the effects of nicotine involves no nicotinic receptors or other mechanisms. In addition, nicotine has also been shown to induce free radical generation and cause lipid peroxidation in pancreatic tissue and esophageal mucosa (Wetscher et al., 1995). Results of the studies demonstrated that incubation of homogenized pancreatic tissue and esophageal tissue with nicotine increased the generation of lipid peroxides. Employment of free radical scavenging enzymes, superoxide dismutase and catalase, along with nicotine abolished the generation of lipid peroxides suggesting a role for superoxide and hydrogen peroxide in nicotine induced oxidative stress. In more recent years, the effects of antioxidant molecules on nicotine induced oxidative stress has been investigated. It was reported that vitamin E prevents the nicotine induced oxidative stress in rat brain (Gumustekin et al., 2003). A preventive effect for vitamin C and S-allylcysteine on nicotine induced oxidative stress is also described (Kelly, 2003; Helen et al., 2003). It is possible to see controversial results on the effects of nicotine on oxidative stress too. In one study, different concentration of nicotine was reported to both induce and prevent oxidative stress. High concentrations of nicotine (1–10 mM) were inductive of oxidative stress whereas low concentration of nicotine (10 µM) was preventive of oxidative stress (Guan et al., 2003). However, in another study it was reported that 0.8 µM of nicotine was sufficient to induce oxidative stress (Crowley-Weber et al., 2003). Prevention of oxidative stress with low concentration of nicotine is suggested to result from inhibition of H₂O₂ induced lipid peroxidation (Guan et al., 2003). This is controversial with previous results which suggested that nicotine induced oxidative stress involves H₂O₂. However, it is in agreement with more recent findings which suggest that nicotine sequesters Fe²⁺ and inhibits the Fenton reaction (Soto-Otero et al., 2002).

1.5.2. The effects of nicotine on apoptosis

It has been shown that nicotine is implicated in prevention of apoptosis (Aoshiba et al., 1996). Nicotine has been reported in previous studies to be implicated in development of human lung cancer (Heusch and Maneckjee, 1998). This study provided evidence that nicotine activates the kinase (ERK2), which results in increased expression of the Bcl-2 oncoprotein and suppression of apoptosis. In the same study, it has also been shown that nicotine blocks the inhibition of PKC and ERK2 activity in lung cancer cells by anticancer agents. These effects of nicotine have been observed to occur at the 1 μ M concentrations or less generally found in the blood of smokers. Another study also described a mechanism for nicotine induced inhibition of apoptosis involving Bcl2 (Mai et al., 2003). They suggested that nicotine might exert a regulatory effect on Bcl2 because nicotine induces extensive Bcl2 phosphorylation. In another study, it has also been shown that nicotine acting through nicotinic acetyl choline receptors suppresses apoptosis (Maneckjee and Minna, 1994). In the same study, it was demonstrated that nicotine blocks the induction of apoptosis induced by opioids such as morphine or methadone. Nicotinic receptor antagonists hexamethonium and decamethonium were able to reverse this process indicating the involvement of nicotinic receptors in suppression of opioid induced apoptosis by nicotine. Nicotine has also been shown to rescue PC12 cells from death induced by nerve growth factor deprivation and to prolong neutrophil survival by suppressing apoptosis (Yamashita and Nakamura, 1996). Serum deprivation generates the death of undifferentiated PC12 cells. This death is inhibited by addition of nerve growth factor. Even after PC12 cells are differentiated, cell death occurs following nerve growth or serum deprivation. The addition of nicotine in serum deprived PC12 cells prevented the cell death. The protective effect of nicotine was reversed by nicotinic receptor antagonists indicating the involvement of nicotinic acetyl choline receptors in the prevention of cell death. Nicotine has also been shown to inhibit the UV-induced apoptosis (Sugano et al., 2001). This inhibition was found to correlate with prevention of cytochrome c release and caspase activation, which are described to be important components of the UV-induced apoptotic pathway. In addition to the inhibitory effects of nicotine on apoptosis, stimulatory effect on apoptosis is also suggested for nicotine in some experimental systems. It was shown that nicotine stimulates the apoptotic pathway possibly through the Hsp 90 alpha expression (Wu et al., 2002).

1.5.3. The effects of nicotine on cell proliferation

It has been shown nicotine imposes a dose dependent inhibition of cell proliferation when added to BALB/C 3T3 cell culture media (Konno et al., 1991). In the same study the effect of nicotine on newly synthesized and secreted proteins was also investigated. The study demonstrated that nicotine suppressed cell proliferation in a concentration

dependent manner and induced significant morphological changes. The study also demonstrated that the inhibitory effect of nicotine is mediated by cell-modulating factors synthesized or secreted by nicotine exposed cells. The described results indicated that nicotine modulates cell proliferation via the synthesis or secretion of heat labile growth modulating factors. Similar results were obtained in another experimental system using HL-60 cells (Konno et al., 1986). Addition of nicotine caused a dose dependent inhibition of cell proliferation. It has also been suggested, in the same study, that anticellular effects of nicotine are accompanied by a significant change in the cell cycle distribution of HL-60 cells. Treatment with 4 mM of nicotine for 20 h caused an increase in the proportion of G1 phase cells and a significant decrease in the amount of S-phase cells. It was also demonstrated that nicotine mainly affects the de novo synthesis of proteins. Contradictory results were observed in another experimental system (Woggner and Wang, 1994). It was shown that nicotine at 100 ng/ml and 10 μ g/ml significantly stimulated epithelial cell growth in two ectocervical and three human papilloma virus DNA treated cell lines (Woggner and Wang, 1994). Cell proliferation, in this system, was inhibited 50% by 100 μ g/ml and 10 mg/ml nicotine. However, these concentrations exceeded the concentrations of nicotine reported in smoker's servical mucus. The inhibitory action of nicotine on apoptosis and the stimulatory action on cell proliferation in some cases may support the hypothesis that nicotine is implicated in carcinogenesis.

1.5.4. Genotoxic potentials of nicotine

Studies also evaluated the genotoxic potentials of nicotine and its major metabolites cotinine, nicotine-*N*-oxide, and *trans*-3'-hydroxycotinine by mutagenicity assay (Doolittle et al., 1995). None of these compounds were able to induce mutations or sister chromatid exchange in this experimental system. However, opposite results were established in another study using Chinese Hamster Ovary cells (Trivedi et al., 1990). Genotoxicity of nicotine was evaluated in Chinese Hamster Ovary cells by the rate of occurrence of sister chromatid exchange and chromosome aberrations. Nicotine was reported to increase chromosome aberrations and sister chromatid exchange frequency in a dose and time dependent manner in Chinese Hamster Ovary cells. It was reported that nicotine was genotoxic at the concentrations found in saliva levels of nicotine achieved during tobacco chewing (Trivedi et al., 1990).

1.5.5. The effects of nicotine on gene expression

It has been shown that nicotine increases catecholamine secretion and activates the enzymes that function in catecholamine biosynthetic pathways such as tyrosine hydroxylase and dopamine β -hydroxylase in adrenal medullar cells (Hiremagular et al., 1993; Sun et al., 2003). The effects of long term exposure to nicotine on tyrosine hydroxylase and dopamine β -hydroxylase gene expression

was investigated. It has been shown that nicotine exposure for 1–2 days increases both tyrosine hydroxylase and dopamine β -hydroxylase mRNA levels. It was suggested that the effect of nicotine was transcriptionally mediated. Deletion of the 5' promoter region of the tyrosine hydroxylase gene showed that the region containing a cAMP/calcium regulatory element is sufficient for the nicotinic induction of tyrosine hydroxylase.

In other studies it was shown that nicotine co-induces the heat shock proteins, HSP70 and HSP28, and causes an elevation of c-fos protooncogene expression (Hahn et al., 1991). Heat shock proteins are expressed in response to a variety of stresses such as exposure to heat and ethanol (Lindquist and Graig, 1988). Heat shock proteins protect the cells against the stressor and many other insults (Gerner and Schneider, 1975). It has been suggested that heat shock proteins also play a role in regulation of cell cycle and during specific stages in the development of the organism. In one study, it was suggested that although nicotine does not directly increase the expression of heat shock proteins, it cooperates with ethanol to increase the synthesis of heat shock proteins (Hahn et al., 1991). The suggested role for nicotine was demonstrated on three levels of investigations: protection against heat, induction of specific gene expression, and binding of heat shock transcription factor to the heat shock element. In another study, it was demonstrated that prenatal nicotine exposure causes elevations of c-fos expression in fetal and neonatal brain. (Slotkin et al., 1997). The expression of c-fos has been recently shown to correlate with cellular thiol levels (Chang et al., 2003). The presence of GSH precursor decreased the c-fos expression by nicotine whereas the presence of an inhibitor of GSH synthesis increased the c-fos expression following nicotine treatment.

1.5.6. The effects of nicotine on the endocrine system

It has been shown that nicotine administration induces changes in hormones associated with the hypothalamic pituitary axis (HPA) (Pickworth and Font, 1998). There is evidence suggesting an interaction between smoking and hormones in the HPA such as adrenocorticotrophic hormone (ACTH) and cortisol (Fuxe et al., 1989). These hormones have been shown to be involved in stress and many of the symptoms of tobacco abstinence. They are also involved in anxiety, restlessness, inability to concentrate, and sleep disturbances (Hughes and Hatsukami, 1986). It has been suggested that hypothalamic activity is involved in the regulation of homeostatic processes. It regulates thirst, hunger, body temperature, respiration, and the sleep-wake cycle (Everitt and Hokfeite, 1989). It has also been suggested that nicotine induced changes in heart rate, blood pressure and peripheral blood flow may also be the result of actions at the hypothalamic site. It has been shown that there are types of nicotine binding sites in the hypothalamus. One is a high affinity but low density binding site, which is widely distributed throughout hypothalamic nuclei and the preoptic

areas. Another one is a low affinity but high density binding site which is widely distributed within the hypothalamus and finally, α -bungarotoxin sites that are located in the hypothalamus and preoptic areas. Nicotine stimulates the release of acetyl choline or monoaminergic neurotransmitters. It has been suggested that nicotine functions at presynaptic neuronal acetyl choline receptors to cause the influx of calcium ion and cause the synaptic release of acetyl choline or glutamate. Endocrine effects of nicotine have been suggested to be caused by actions at postsynaptic cholinergic binding sites that directly regulate releasing factor excretion or through presynaptic sites on monoaminergic neurons that modulate releasing factor excretion. Administration of nicotine through smoking has been shown to increase the level of ACTH (Seyler et al., 1984). Intense smoking was necessary for an increase in cortisol levels. It has been shown that following intense smoking plasma levels of β -endorphin and vasopressin were significantly increased (Millane et al., 1984). Intravenous nicotine administration also increased the ACTH and cortisol levels suggesting that it is nicotine not other components of tobacco smoke that increase ACTH and cortisol levels. It has also been shown that nicotine administration increases plasma levels of vasopressin (Millane et al., 1984).

1.6. Differential actions of nicotine enantiomers

Enantiomers of nicotine have been shown to display different effects on targets. There have been many studies comparing the toxicities, receptor binding capacities, ability to increase the blood pressure and differential metabolism of the nicotine enantiomers (Pictet and Rotschy, 1904; Hicks et al., 1947; Abood et al., 1985; Martin et al., 1983; Nwosu and Crooks, 1988). It has been found that in animals (–)-nicotine appeared to be about twice as toxic as (+)-nicotine (Pictet and Rotschy, 1904). It has also been shown that administration of (–)-nicotine generated convulsion whereas (+)-nicotine caused twitching of the skin (Pictet and Rotschy, 1904). In a study that compared the effectiveness of the nicotine enantiomers, it was found that (–)-nicotine was more efficient in increasing the blood pressure in animals. In another study it was demonstrated that both stereo-isomers of nicotine have antinociceptive effects (Monser and Matilla, 1975; Tripathi et al., 1982). However, the antinociceptive effects of (–)-nicotine were 970 times more potent than the antinociceptive effect of (+)-nicotine. Previous studies used antagonists to explore receptors involved in antinociceptive effects of nicotine. Mecamylamine, which inhibits nicotinic receptors, was also found to antagonize effectively the antinociceptive actions of (–)-nicotine suggesting that nicotinic receptors are involved in antinociception. The binding of (–)-nicotine and (+)-nicotine to rat brain membranes has also been investigated (Abood et al., 1985). Studies revealed that only the (–)-nicotine showed high affinity binding; while the (+)-nicotine was at least 1/10 as effective as

the (–)-nicotine when in competition with (+)-nicotine as the ligand. The oxidative stress inducing capacity of both (–)- and (+)-nicotine was also compared and found that (–)-nicotine is more potent in induction of oxidative stress compared to (+)-nicotine (Yildiz et al., 1988).

1.7. Nicotine and addiction

The addiction process is suggested to begin with interaction of nicotine with nicotinic acetyl choline receptors (nAChRs). This interaction is proposed to cause the activation of the reward centers in the central nervous system (Mansvelder and McGehee, 2002). Nicotinic cholinergic receptors are ligand-gated ion channels composed of five subunits (Vidal, 1996). These subunits are 2α , 1β , 1δ and 1γ . Nicotinic receptors are widely distributed in the brain. In the brain and ganglia, neuronal nicotinic receptors are composed of only α and β subunits. The distribution and combination of subunits may vary from species to species. Researchers demonstrated that there are two main binding sites in the brain for nicotine (Wada et al., 1989). These are one high affinity site composed of an $\alpha 4 \beta 2$ combination, and one low affinity site probably composed of $\alpha 7$ subunit. The low affinity site is antagonized by α -bungarotoxin. The high affinity site is antagonized by mecamylamine. It has been shown that nicotine binds to its receptors on cell bodies at nerve terminals. It is proposed that nicotine may play a role in modulating the release of neurotransmitters such as acetyl choline, nor epinephrine, dopamine, serotonin and glutamate through presynaptic nicotinic receptors (Gray et al., 1996). The main reinforcing effect of nicotine could be suggested to be dependent on the effect of nicotine on dopaminergic system (Vezina et al., 1991). It has been shown that nicotine exposure results in generation of locomotor stimulant effect originating from dopaminergic mechanisms. These effects of nicotine have been shown to disappear when dopaminergic neurons are damaged (Clarke, 1991). Evidence has also been presented that A10 neurons and neurons of the ventral tegmental area are implicated in the psychostimulant effects of nicotine. The effect of haloperidol on nicotine intake was also investigated (Dave et al., 1995). It was shown that following haloperidol administration nicotine intake was increased. These results may indicate that blockade of dopaminergic receptor decreases the drug reward which leads to increased nicotine intake to maintain satisfaction.

1.8. Beneficial effects of nicotine

Nicotine, the major alkaloid in tobacco, has been shown to cause several types of illness and death of million of people. On the other hand, nicotine has also been proven to be a pharmaceutical agent (Jarvik, 1991). Smokers become addicted to nicotine for one reason, which is pleasure (Wilbert, 1987). In this sense, the principal reason for nicotine use is its ability to make people feel good.

In a study, the psycho physiological effects of nicotine abstinence were investigated. It was reported that abstinence of nicotine was altering the mood and performance in a negative way (al'Absi et al., 2002). It has been suggested that reward mechanisms play an essential role in learning (Greenhoff et al., 1986). Evidence indicates that nicotine stimulates dopaminergic neurons in the mesolimbic system and releases dopamine in these reward centers. It has also been shown that nicotine facilitates electrical brain stimulation of brain reward areas in rats (Bolofovski and Dumery, 1987). In recent study nicotine treatment was demonstrated to improve reward related learning in rats (Olausson et al., 2003). The ability of nicotine to relieve stress and anxiety is still a subject of research. Nicotine has long been suggested to have anxiolytic effect, which is proposed to occur through the action of nAChRs. Evidence is presented recently supporting the involvement of these receptors in relieve of anxiety (Salas et al., 2003). In a few other former investigations, it was demonstrated that increased smoking during stressful conditions induces anxiety reduction (Nesbitt, 1973). It is suggested that nicotine reduces body weight by at least two mechanisms (Grunberg et al., 1984). It can reduce appetite and increase the metabolic rate. Nicotine induces serotonin release, which causes weight loss. These evidences suggest that stopping nicotine intake results in weight gain by decreased metabolism and increased eating. A few experimental systems demonstrated that smokers feel that smoking helps them to concentrate (Warburton and Walters, 1989). It was shown that increasing the plasma nicotine level of non-deprived smokers with nicotine gum improved speed and accuracy on a number of psychometric investigations (Hidmarch et al., 1990). Non-smokers given the same procedure did not show any significant effect of the drug. In contrast to human studies, animal studies showed stronger evidence of facilitation of performance, learning, and memory. Smoking has been shown to be associated with the occurrence of many fatal diseases. However, the possibility exists that nicotine also could be used in protection against a number of diseases with relatively low frequencies. It has been demonstrated that in previous studies that smokers develop Parkinson's disease with relatively low frequencies (Reavill, 1990). Another disorder, Tourette's syndrome appears to be nicotine sensitive (Devor and Izenberg, 1989). It has been shown that nicotine potentiates the effects of haloperidol in tourette's patients (Sanberg et al., 1989). Another disease that is suggested to be sensitive to nicotine is ulcerative colitis (Loshner et al., 1990).

2. Conclusion

Research on nicotine has been going on for about two centuries. Since then a substantial amount of work has been carried out by several researchers on different aspects of

nicotine. The extensive studies on nicotine metabolism have almost clarified the pathways taken by nicotine and the enzymes involved in these pathways. However, all of the enzymes involved in nicotine metabolism have not yet been completely identified and characterized therefore more research will be needed in this direction. Investigation of genetic polymorphism in nicotine metabolism revealed the characteristics of interindividual differences in nicotine response such as nicotine intake, addiction and induction of lung cancer. Now it seems that the rate of nicotine intake and the individual response to nicotine intake is largely determined by the nature and the activity of nicotine metabolizing enzymes. Association of CYP2A6 gene deletion with low risk of cancer may explain a mechanism by which tobacco smoke and nicotine can cause cancer in smoking individuals. Inhibition of apoptosis that largely eliminates nascent cancer cells and stimulation of cell proliferation in some experimental systems by nicotine may also contribute to the nicotine induced carcinogenesis. However, in order to be able to have an in depth look at the exact mechanism by which nicotine induces cancer more research related to its metabolism, cell proliferation, and apoptosis will be needed. Since its discovery nicotine has been found to be implicated in several biological processes in most of which displaying a dual effect and therefore making the elucidation of its exact role more difficult. Investigations explaining the controversial actions of nicotine exist and are continuing to accumulate especially in areas such as oxidative stress, apoptosis, and cell proliferation. Induction of oxidative stress by nicotine generally occurs at higher concentrations whereas at lower concentrations it seems to inhibit oxidative stress. Less frequency occurrence of Parkinson's diseases, which is characterized by enhanced oxidative stress in smoking people, may thus be attributed to nicotine's inhibitory effect on oxidative stress. More studies will still be needed to clarify the biological effects of nicotine in different experimental systems.

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