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Nicotine promotes cell proliferation via α7-nicotinic acetylcholine receptor and catecholamine-synthesizing enzymes-mediated pathway in human colon adenocarcinoma HT-29 cells

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Abstract

Cigarette smoking has been implicated in colon cancer. Nicotine is a major alkaloid in cigarette smoke. In the present study, we showed that nicotine stimulated HT-29 cell proliferation and adrenaline production in a dose-dependent manner. The stimulatory action of nicotine was reversed by atenolol and ICI 118,551, a β_1- and β_2-selective antagonist, respectively, suggesting the role of β-adrenoceptors in mediating the action. Nicotine also significantly upregulated the expression of the catecholamine-synthesizing enzymes [tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DβH) and phenylethanolamine *N*-methyltransferase]. Inhibitor of TH, a rate-limiting enzyme in the catecholamine-biosynthesis pathway, reduced the actions of nicotine on cell proliferation and adrenaline production. Expression of α7-nicotinic acetylcholine receptor (α7-nAChR) was demonstrated in HT-29 cells. Methyllycaconitine, an α7-nAChR antagonist, reversed the stimulatory actions of nicotine on cell proliferation, TH and DβH expression as well as adrenaline production. Taken together, through the action on α7-nAChR nicotine stimulates HT-29 cell proliferation via the upregulation of the catecholamine-synthesis pathway and ultimately adrenaline production and β-adrenergic activation. These data reveal the contributory role α7-nAChR and β-adrenoceptors in the tumorigenesis of colon cancer cells and partly elucidate the carcinogenic action of cigarette smoke on colon cancer.

Keywords: Adrenaline; α7-Nicotinic acetylcholine receptor; β-Adrenoceptors; Colon cancer; Nicotine

Introduction

Cigarette smoking is a serious issue in public health and is responsible for millions of death worldwide (Batra et al., 2003). Cigarette smoke contains more than 3000 chemicals. About 60 components in cigarette smoke were identified as carcinogens

Table 1
Oligonucleotide sequences of primers used for RT-PCR

Name	Primer sequence	Fragment size (bp)
TH	Sense: CGAGCTGTGAAGGTGTTTGA Antisense: GCTGGGGGATATTGTCTTCC	523
AADC	Sense: AGGAAGCCCTGGAGAGAGAC Antisense: TTCAGGTAAGTGGGGTCCAG	333
DβH	Sense: CCATCAGCATTCAGGGCTTA Antisense: CCACAGACAGCTGAGTTCCA	544
PNMT	Sense: GCCACCGGTGAAGTGTCC Antisense: CTTGTAGCCACTACGCACCA	552
α7-nAChR	Sense: GGCCAATGACTCGCAACCACT Antisense: GACCAGCCTCCGTAAGACCAG	399
β-Actin	Sense: AGAAAATCTGGCACCACCACC Antisense: GTACTTGCGCTCAGGAGGAG	764

Abbreviations: AADC, aromatic-L-amino acid decarboxylase; DβH, dopamine-β-hydroxylase; PNMT, phenylethanolamine *N*-methyltransferase; RT-PCR, reverse transcription-polymerase chain reaction; TH, tyrosine hydroxylase.

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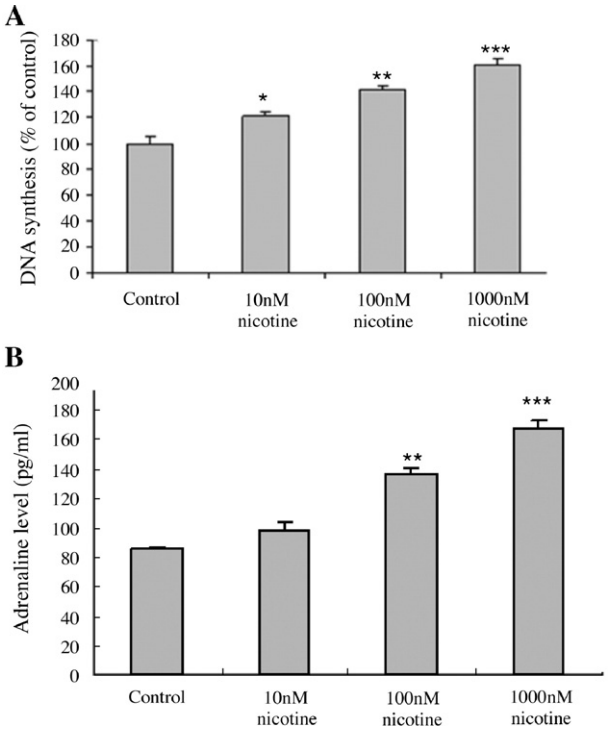

Fig. 1. Effects of nicotine on HT-29 cell proliferation and adrenaline production after incubation for 5 h at the doses of 0–1000 nM. (A) Effect of nicotine on HT-29 cell proliferation. Incubation with nicotine increased cell proliferation in HT-29 cells in a dose-dependent manner. (B) Effect of nicotine on adrenaline production in HT-29 cells. Incubation with nicotine increased adrenaline production in HT-29 cells in a dose-dependent manner. Results were expressed as the mean±SE ($n=3$). *$p<0.05$; **$p<0.005$; ***$p<0.0005$, significantly different from the control group.

such as polycyclic aromatic hydrocarbons, heterocyclic hydrocarbons and *N*-nitrosamines. Nicotine, a major active component of cigarette smoke, plays a dominant role in mediating the biochemical, pharmacological and psychological effects of cigarette smoking. Nicotine is known to be highly addictive (Hecht, 1999). Recent studies also suggested that nicotine might contribute directly to tumor initiation resulting from smoking (Kleinsasser et al., 2006). Nicotine has been implicated in the growth promotion of various cancers. More recent data have suggested that nicotine may add to the cancer risk by stimulating cell growth, suppressing apoptosis and by inducing angiogenesis (Heusch and Maneckjee, 1998; Natori et al., 2003; Mousa and Mousa, 2006).

Colon cancer is the leading cause of cancer deaths in the Western world (Jemal et al., 2006) and smoking is a putative risk factor for colon cancer (Sturmer et al., 2000; Giovannucci, 2001). Recent studies suggested that smokers have a higher risk for developing colon cancer than non-smokers (Ulrich et al., 2001; Michael et al., 2002; Slattery et al., 2003). However, the molecular and cellular mechanisms by which smoking increases risk of colon cancer remain understudied.

Adrenaline has been shown to play a contributory role in cancer growth (Lutgendorf et al., 2003). The catecholamine-synthesizing enzymes namely tyrosine hydroxylase (TH), aromatic-L-amino acid decarboxylase (AADC), dopamine-β-hydroxylase (DβH) and phenylethanolamine *N*-methyltransferase (PNMT) are important in the formation of adrenaline (Nagatsu, 1991). Previous studies have suggested that nicotine can regulate the expression of catecholamine-synthesizing enzymes both in normal and cancer cells (Hiremagalur et al., 1993; Craviso et al., 1995; Gueorguiev et al., 1999). However, the growth regulation of colon cancer cells by nicotine and the involvement of catecholamine-synthesizing pathway and adrenaline are largely unknown. All these could be modulated by nicotinic receptors, if any in cancer cells. Based on the above observations, we aimed to delineate the actions of nicotine on colon cancer growth and the participation of the α7-nicotinic acetylcholine receptor (α7-nAChR), catecholamine-synthesizing enzymes and adrenaline in this biological process.

Materials and methods

Reagents and drugs. Nicotine, atenolol (β_1-selective antagonists), ICI 118,551 (β_2-selective antagonists), 3-iodotyrosine (TH inhibitor), methyllycaconitine (α7-nAChR antagonist) and antibody for β-actin were purchased from Sigma (St. Louis, MO, USA). Antibody for TH was purchased from Santa

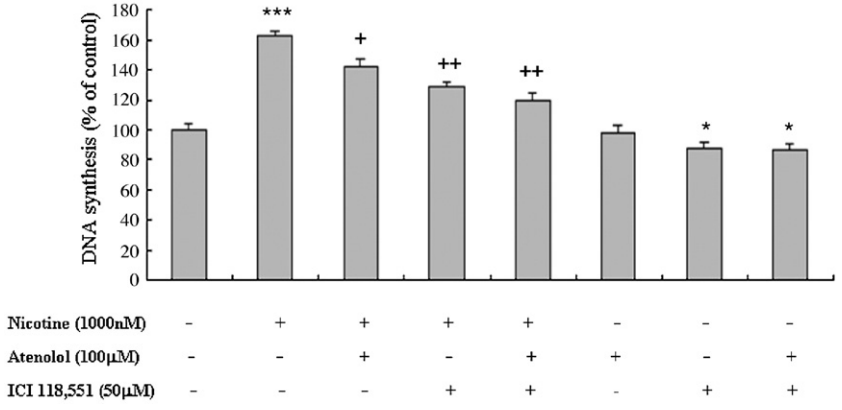

Fig. 2. Effects of β_1- or β_2-adrenoceptor blockade with atenolol (100 μM) or ICI 118,551 (50 μM) respectively on nicotine-induced HT-29 cell proliferation. Pretreatment with β_1- or β_2-selective antagonists for 45 min before incubation with nicotine for 5 h reversed the nicotine-induced cell proliferation. Results were expressed as the mean±SE ($n=3$). *$p<0.05$; ***$p<0.0005$, significantly different from the control group. $^{+}p<0.05$; $^{++}p<0.005$, significantly different from the nicotine-treated group.