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The involvement of AMP-activated protein kinases in the anti-inflammatory effect of nicotine in vivo and in vitro

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ABSTRACT

AMP-activated protein kinase (AMPK) is the downstream component of a kinase cascade that plays a pivotal role in energy homeostasis. AMPK has recently emerged as an attractive and novel target for inflammatory disorders. Thus, the aim of this study was to assess the role of AMPKα in the anti-inflammatory effect of nicotine in carrageenan-induced rat paw edema model and to evaluate the mechanism of nicotine-induced AMPKα phosphorylation in RAW 264.7 cells. The results indicate that nicotine alleviated paw edema and the activation of AMPKα involved in the anti-inflammatory effect of nicotine in vivo. In addition, nicotine was able to activate AMPKα phosphorylation in macrophages and this effect was mediated through nicotinic acetylcholine receptors. Furthermore, nicotine significantly induced the phosphorylation of Akt and the Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) protein expression in macrophages. Wortmannin, a specific inhibitor of phosphotidylinositol 3-kinase (PI3K), suppressed nicotine-induced Akt and AMPKα phosphorylation. STO-609, a CaMKK inhibitor, not only inhibited the activation of AMPKα but also suppressed the phosphorylation of Akt induced by nicotine. In conclusion, both of CaMKK and PI3K/Akt pathways are involved in the nicotine-induced AMPKα phosphorylation in macrophages, and the interaction of CaMKK and Akt may exist. AMPKα is a novel and critical component of anti-inflammatory effect of nicotine.

1. Introduction

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase composed of a catalytic α subunit and regulatory β and γ subunits [1]. AMPK has been shown to function as a sensor of the energy state of the cell. When the AMP/ATP ratio increases, AMPK is activated, and a conformational change is induced by combining with AMP, thereby decreasing the AMP/ATP ratio by switching off ATP-consuming pathways and switching on ATP-generating pathways [2]. Additionally, AMPK acts as a regulator of the whole-body energy metabolism by mediating the effects of hormones such as adiponectin, leptin, or ghrelin [3].

AMPK activation requires phosphorylation of threonine 172 in the activation loop of α subunit [4]. Two AMPK activating kinases have been identified recently. LKB1, a tumor suppressor kinase, in complex with two accessory subunits, Ste20-related adaptor protein (STRAD) and MO25, has been shown to phosphorylate AMPK [5]. Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) has also been identified as an AMPK kinase [6]. In addition to phosphorylation, AMPK is allosterically activated by binding of AMP, and this can also

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promote phosphorylation of threonine 172 [7]. However, AMPK can be activated in an AMP-independent manner as shown with hyperosmotic stress or with the anti-diabetic drug metformin [8]. The finding that CaMKK acts upstream of AMPK suggests that in addition to changes of the AMP/ATP ratio, an increase of intracellular Ca^{2+} acts as a second pathway to activate AMPK.

Although the actions of AMPK were initially defined as regulation of fatty acid and cholesterol synthesis pathways, several studies have suggested that AMPK plays an important role in the treatment of type 2 diabetes mellitus [9], protection from apoptosis [10], regulation of angiogenesis [11] and exerts anti-atherosclerotic effects [12]. More recent studies also suggest AMPK is involved in anti-inflammatory signaling [13].

Nicotine, a nicotinic cholinergic agonist, binds and activates nicotinic acetylcholine receptor (nAChR) [14] and exerts anti-inflammatory activities in vitro and in vivo [15,16]. Nicotine has been shown to be an effective treatment in experimental animal models of inflammatory bowel disease [17] and sepsis [18]. The molecular mechanisms responsible for the anti-inflammatory effects of nicotine are currently being assessed.

Therefore, the present study was undertaken in vivo and in vitro, to evaluate whether AMPK is involved in the anti-inflammatory effect of nicotine and to investigate the signaling mechanism leading to AMPK activation.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats weighing 300–400 g were purchased from the National Laboratory Animal Breeding and Research Center of the National Science Council, Taiwan. All animals were housed at an ambient temperature of 23 ± 1 °C and humidity of $55 \pm 5\%$ with 12 h light/dark cycle and had free access to food and water.

2.2. Carrageenan-induced paw edema

Paw edemas were induced by subcutaneous injection of 100 μl of 1% lambda carrageenan solution (Sigma) (w/v solution in saline, 0.9% NaCl) in the plantar aponeurosis of the right hind paw. Carrageenan is a sulfated polysaccharide that promotes acute inflammation by activating proinflammatory cells [19]. Nicotine (0.1 ml, 1 mg/kg), diluted in saline solution, was given intraplantar (i.pl.) injection into right hind paw at 30 min before carrageenan injection. In the antagonism studies, compound C ((6-[4-(2-piperidin-1-yl-ethoxy)-phenyl])-3-pyridin-4-yl-pyyrazolo [1,5-a] pyrimidine) (Merck: 0.1 ml, 1.5 mg/kg), an inhibitor of AMPK [20], was given intraplantar injection 30 min before nicotine. An equal volume of the vehicle (saline) was given to the control group. The volume of the paw was measured by a volume displacement method using a digital plethysmometer (Ugo Basile, Comerio VA, Italy) prior to any treatment, and at 3 h after carrageenan administration. The increase in volume and the percent change caused by the irritant were estimated after subtracting the basal volume of the paw before injection. A lower numerical value (in percent) would indicate stronger anti-inflammatory activity.

2.3. Tissue preparation

The animals were killed with a lethal injection of sodium pentobarbital (50 mg/kg, i.p.) at 3 h after carrageenan injection, paws were cut at the level of the calcaneus bone, weighed and the tissues were frozen at −80 °C until processed.

2.4. Protein purification

The samples were ground in a mortar containing liquid nitrogen. The powered tissue was suspended in 1 ml of lysis buffer (Tris–HCl 50 mM, EDTA 1 mM, dithiothreitol 1 mM, 1% Triton X-100) containing protease inhibitors (leupeptin 10 mM, aprotinin 10 mM, pepstatin A 1 mM, and phenylmethylsulfonyl fluoride (PMSF) 10 mM) and agitated at 4 °C for 1 h. After centrifugation at 10,000 × *g* at 4 °C for 30 min, the protein concentration in the supernatant was determined using BCA protein assay kit (Pierce, Rockford, IL, USA). The supernatant was stored at −80 °C until iNOS and AMPK assay.

2.5. Determination of tumour necrosis factor α (TNF-α) production in paw

Frozen tissue samples were weighed and placed in lysis buffer at a ratio of 100 mg/ml of buffer. Tissue supernatants were analyzed for TNF-α using ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions.

2.6. Cell culture

RAW264.7, a monocyte-macrophage cell line, was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM glutamine, antibiotics (100 U/ml of penicillin A and 100 U/ml of streptomycin), and 10% heat-inactivated fetal bovine serum (Gibco/BRL) and maintained in a 37 °C humidified incubator containing 5% CO_2 atmosphere.

2.7. Cell viability assay

Cell viability was measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazolium bromide] assay [21]. MTT (100 μg/ml, Sigma) was added to the medium in each well, and plates were incubated for 3 h at 37 °C. Medium was then removed, and dimethyl sulfoxide (DMSO, 200 μl) was added to each well to solubilize the purple formazan crystals created by mitochondrial dehydrogenase reduction of MTT. After 5 min of additional incubation, absorbance was read at 550 nm on a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The data were expressed as percent cell viability compared with control (DMSO).

2.8. Western blot analysis

Cellular proteins were extracted from control and treated RAW 264.7 cells. Washed cell pellets were resuspended in

extraction lysis buffer [50 mM HEPES (pH 7.0), 250 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5 mM dithiothreitol, 5 mM Na fluoride (NaF), 0.5 mM Na orthovanadate] containing 5 mg/ml of each of leupeptin and aprotinin, and then incubated for 30 min at 4 °C. Cell debris was removed by microcentrifugation, and supernatants were quick frozen. The protein concentration was determined using BCA protein assay kit (Pierce) according to the manufacture's instruction.

Samples containing equal amounts of protein were loaded onto 10% or 7.5% sodium dodecyl sulfate-polyacrylamide gels, subjected to electrophoresis, and subsequently blotted onto nitrocellulose membrane (Millipore, Bedford, MA, USA). Membranes were blocked with Tris-buffered saline (TBS) buffer, pH 7.4, containing 0.1% Tween 20 and 5% skim milk and then incubated overnight at 4 °C with various primary antibodies in TBS containing 0.1% Tween 20. The antibodies included rabbit anti-phosphorylated-Akt, rabbit-anti-Akt, rabbit anti-phosphorylated AMPKα, rabbit anti-AMPKα, rabbit anti-LKB1, rabbit anti-iNOS (1:1000 dilution, Cell Signaling Technology, MA, USA), rabbit anti-CaMKK (1:1000, BD Transduction Laboratories, KY, USA), and mouse anti-β-actin (1:2000 dilution; Sigma–Aldrich, St. Louis, MO, USA). The membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:1000 dilution, Cell Signaling). The blots were detected with an enhanced chemiluminescence kit (Pierce), and the membranes were exposed to X-ray film (Kodak, Rochester, NY, USA) for 5 min. The density of the respective bands was quantified by densitometric scanning of the blots using Image-Pro software (Media Cybermetrics, Inc.).

2.9. Statistical analysis

Data are expressed as means ± S.E.s. One-way ANOVA was performed for the statistical analysis of data; when group comparisons showed a significant difference, the Student–Newman Keuls test was used. $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Inhibitory effect of nicotine on carrageenan-induced edema in rats

To investigate the effect of nicotine on carrageenan-induced edema in rats, injection of carrageenan into the hind paw of rats resulted in a rapid and marked increase in paw volume as a consequence of edema formation (Fig. 1A). The increase in paw volume could be significantly reduced by pre-treatment with nicotine (1 mg/kg, intra-plantar) (from 88.5 ± 3.2% to 30.5 ± 1.3%). In contrast, compound C ((6-[4-(2-piperidin-1-yl-ethoxy)-phenyl])-3-pyridin-4-yl-pyyrazolo [1,5-*a*] pyrimidine) (1.5 mg/kg, intra-plantar; an AMPK inhibitor) dramatically attenuated the inhibitory effect of nicotine on carrageenan-induced edema (from 30.5 ± 1.3% to 92.3 ± 1.9%).

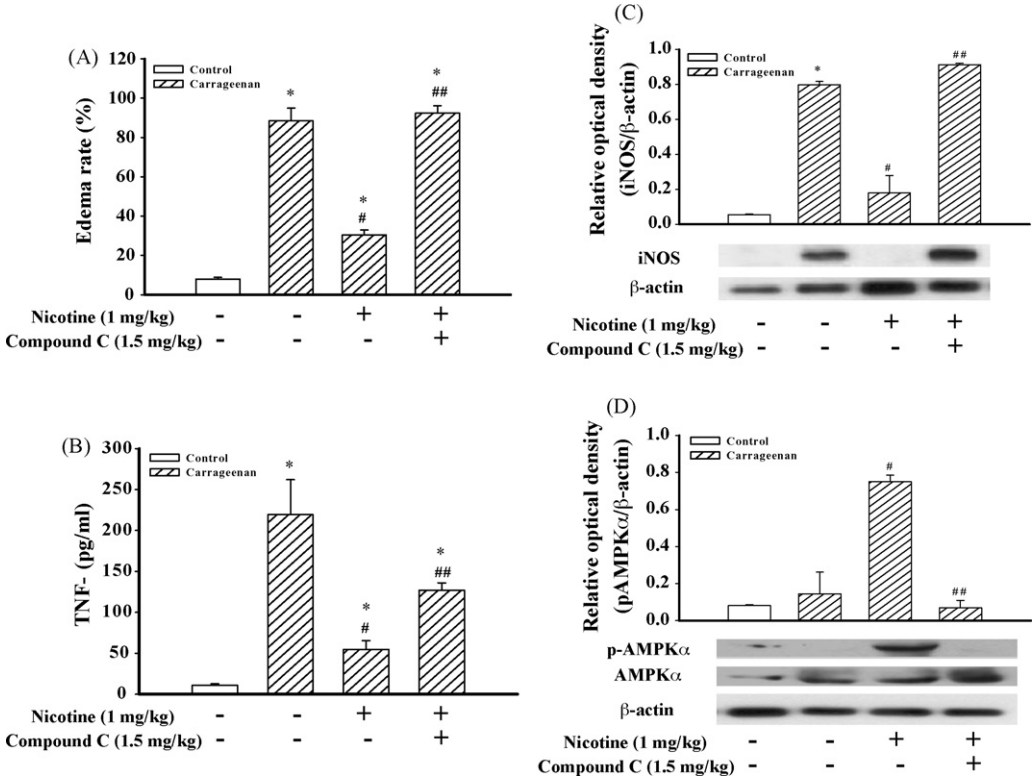

Fig. 1 – Effects of nicotine (1 mg/kg; i.pl.) and compound C (1.5 mg/kg, i.pl.) on carrageenan-induced edema (A), the levels of TNF-α production (B), the expression of iNOS protein (C), and the phosphorylation of AMPKα (D) in rat pads. Data are shown as means ± S.E.s ($n = 6$). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. carrageenan alone, $^{\#\#}P < 0.05$ vs. nicotine + carrageenan.

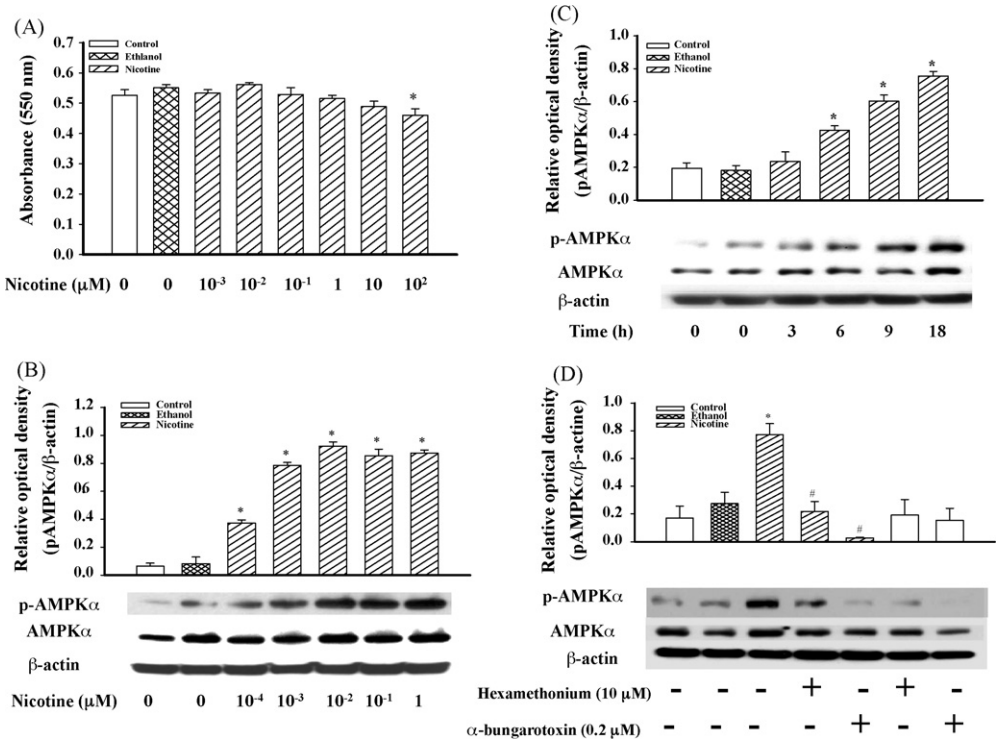

Fig. 2 – (A) Effects of nicotine on macrophage viability. RAW 264.7 cells were exposed to nicotine (10^{-3} μM to 10^{2} μM) for 18 h. Cell viability was determined by the MTT assay as described in the Materials and Methods. Data are shown as means ± S.E.s ($n = 6$). (B) Effects of nicotine (10^{-4} μM to 1 μM) on AMPKα phosphorylation in RAW 264.7 cells. (C) The effects of nicotine (10^{-3} μM) on the time course of changes in the phosphorylation of AMPKα in RAW 264.7 cells. (D) Modulation of nicotine (10^{-3} μM)-induced AMPKα phosphorylation by nACh receptor blockers, hexamethonium (10 μM) and α-bungarotoxin (0.2 μM), in RAW 264.7 cells. Data are shown as means ± S.E.s ($n = 6$). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. nicotine.

3.2. *Nicotine suppressed the production of TNF-α found in paws*

The TNF-α level of paw dramatically increased after carrageenan challenge. The increase in TNF-α level of paw was significantly reduced by pre-treatment with nicotine (from 219.3 ± 4.2 to 54.68 ± 10.8 pg/ml). In contrast, compound C significantly reversed the inhibitory effect of nicotine on the production of TNF-α (from 54.68 ± 10.8 to 127.1 ± 8.9 pg/ml) (Fig. 1B).

3.3. *Nicotine suppressed the expression of iNOS protein found in paws*

The iNOS protein expression of paw dramatically increased after carrageenan challenge. The increase in iNOS protein expression of paw was significantly reduced by pre-treatment with nicotine. In contrast, compound C significantly reversed the inhibitory effect of nicotine on the expression of iNOS protein (Fig. 1C).

3.4. *Nicotine-induced the phosphorylation of AMPKα in vivo*

The phosphorylation of AMPKα significantly increased after nicotine and carrageenan challenge. In contrast, compound C abrogated the phosphorylation of AMPKα induced by nicotine (Fig. 1D).

3.5. *Effects of nicotine on cultured cell viability*

To exclude any possible interference, the effects of nicotine (10^{-3} μM to 10^{2} μM) on cell viability were tested on RAW 264.7 cells. The survival of cells was not influenced by nicotine (10^{-3} μM to 10 μM) or vehicle (ethanol, <0.01%) for 18 h, however when the concentration of nicotine was reached to 10^{2} μM, the cell viability was significantly decreased (Fig. 2A).

3.6. *Activation of AMPKα by nicotine in RAW 264.7 cells*

To determine whether nicotine induced activation of AMPKα, RAW 264.7 cells were treated with various concentration of nicotine (10^{-4} μM to 1 μM) for 18 h. Phosphorylated AMPKα protein expression in the cell lysate was detected by Western blot. As shown in Fig. 2B, treatment with nicotine (10^{-4} μM) significantly increased the phosphorylation of AMPKα, and reached maximum levels of AMPKα expression when treated with higher concentrations (10^{-2} μM). Furthermore, treatment of cells with nicotine (10^{-3} μM) resulted in time-dependent activation of AMPK, beginning after 6 h incubation and reaching a maximum after 18 h (Fig. 2C). Ethanol (the vehicle

of nicotine, <0.01%) alone had no significantly effect on the activation of AMPKα in RAW 264.7 cells (Fig. 2B and C).

3.7. Nicotine induced the activation of AMPKα via nACh receptor

We studied the effects of pre-treatment with non-specific nACh receptor blocker hexamethonium and specific α7-nACh receptor blocker α-bungarotoxin [22] for 30 min on AMPKα phosphorylation induced by nicotine. As shown in Fig. 2D, hexamethonium (10 μM) or α-bungarotoxin (0.2 μM) completely blocked the activation of AMPKα induced by nicotine (10^{-3} μM) in RAW 264.7 cells.

3.8. Activation of Akt by nicotine in RAW 264.7 cells

To clarify whether the signaling pathway of nicotine-induced phosphorylation of AMPKα was mediated through protein kinase B (Akt), RAW 264.7 cells were treated with nicotine (10^{-3} μM) for 18 h, and the cell extracts were analyzed for phosphorylated and total Akt by Western blotting. Fig. 3A showed the Western blot images of phosphorylated Akt protein. Ethanol (the vehicle of nicotine, <0.01%) alone had no significantly effect on the phosphorylation of Akt in RAW 264.7 cells. Stimulation of RAW 264.7 cells with nicotine increased the phosphorylation of Akt, which was abrogated by pre-treatment with hexamethonium (10 μM) or α-bungarotoxin (0.2 μM).

3.9. Involvement of PI3K/Akt pathway in the activation of AMPKα by nicotine in RAW 264.7 cells

To address the role of PI3K/Akt pathways in the activation of AMPKα by nicotine, we evaluated the effects of wortmannin, a specific inhibitor of PI3K [22], on the phosphorylation of Akt and AMPKα by nicotine. RAW 264.7 cells were pre-treated with wortmannin (20 nM) for 30 min and treated with nicotine (10^{-3} μM) for 18 h. As shown in Fig. 3B and C, wortmannin significantly blocked the phosphorylation of Akt and AMPKα induced by nicotine in RAW 264.7 cells.

3.10. CaMKK mediated AMPKα activation upon nicotine stimulation

To determine whether nicotine is able to activate LKB1 and/or CaMKK in RAW 264.7 cells, cells were exposed to nicotine (10^{-3} μM) for 18 h, and LKB1 and CaMKK protein expression were analyzed by Western blot. RAW 264.7 cells exposed to nicotine exhibited a relative increase (3-fold) in CaMKK protein expression as compared to control cells (Fig. 4B). However, there were no significant differences in LKB1 protein expression between the nicotine-treated and control cells (Fig. 4A). Ethanol (the vehicle of nicotine, <0.01%) alone had no significantly effect on the expression of LKB1 and CaMKK in RAW 264.7 cells. Pre-treatment with hexamethonium or α-bungarotoxin significantly abrogated the expression of CaMKK induced by nicotine in RAW 264.7 cells. Meanwhile, inhibition of CaMKK by STO-609 (5 μM) [6] abolished the phosphorylation of AMPKα and Akt induced by nicotine (Fig. 4C and D).

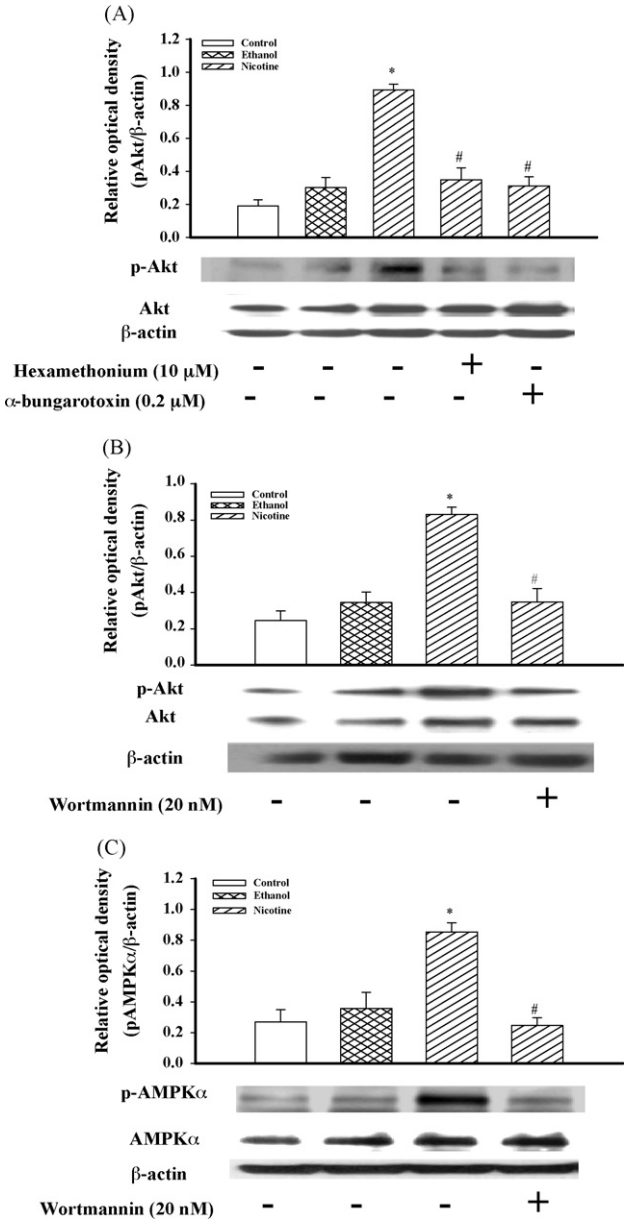

Fig. 3 – The PI3K/Akt pathway is involved in the nicotine-induced AMPKα phosphorylation in RAW 264.7 cells. (A) The effects of nicotine (10^{-3} μM) on the phosphorylation of Akt in RAW 264.7 cells. Data are shown as means ± S.E.s (n = 6). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. nicotine. (B) The modulation of nicotine-induced Akt phosphorylation by wortmannin (20 nM), a PI3K inhibitor, in RAW 264.7 cells. (C) The modulation of nicotine-induced AMPKα phosphorylation by wortmannin in RAW 264.7 cells. Data are shown as means ± S.E.s (n = 6). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. nicotine.

4. Discussion

The present study is the first investigation to demonstrate the activation of AMPKα is involved in the anti-inflammatory effect of nicotine in vivo and in vitro. In RAW 264.7 cells, we demonstrated that nicotine activated the nACh receptor to

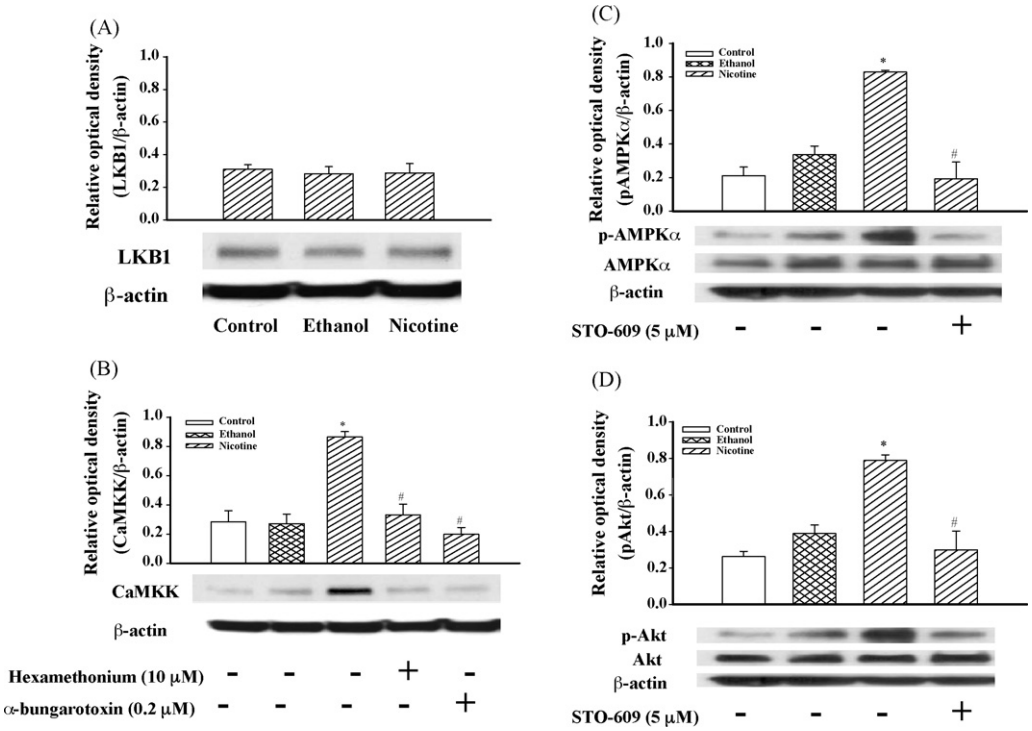

Fig. 4 – The CaMKK is involved in the nicotine-induced AMPKα phosphorylation in RAW 264.7 cells. (A) The effect of nicotine (10^{-3} μM) on the expression of LKB1 in RAW 264.7 cells. (B) The effect of nicotine on the expression of CaMKK in RAW 264.7 cells. (C) The modulation of nicotine-induced AMPKα phosphorylation by STO-609 (5 μM), a CaMKK inhibitor, in RAW 264.7 cells. (D) The modulation of nicotine-induced Akt phosphorylation by STO-609 in RAW 264.7 cells. Data are shown as means ± S.E.s ($n = 6$). *$P < 0.05$ vs. control, #$P < 0.05$ vs. nicotine.

lead to AMPKα phosphorylation. Meanwhile, both of the CaMKK and PI3K/Akt pathways may be associated with the nicotine-induced AMPKα phosphorylation.

The anti-inflammatory effects of nicotine have been previously reported: nicotine inhibits cytokine production [15,17], inhibits NF-κB activation [23], abrogates T cell development and maturation [24], and inhibits neutrophil and monocyte killing function [25]. In animal models, nicotine suppresses the progression of experimental ulcerative colitis [17] and cutaneous inflammation [26] and improves survival during endotoxemia and sepsis [18]. In addition, nicotine has been used successfully in the treatment of human ulcerative colitis [27]. However, the precise mechanism by which nicotine inhibits inflammation in these models is not completely understood. Carrageenan-induced local inflammation in the rat paw is a classical model of edema formation and hyperalgesia that has been extensively used in the development of non-steroidal anti-inflammatory drugs (NSAIDs) [19,28,29]. In the present study, intra-plantar injection of carrageenan resulted in inflammation as identified by rat paw edema and an increase in tissue TNF-α production and iNOS protein expression. Results as shown in Fig. 1A, B and C, pre-treatment with nicotine significantly reduced the level of carrageenan-induced rat paw edema, TNF-α production, and iNOS protein expression. The activation of AMPKα also had been observed when pre-treated with nicotine (Fig. 1D). Meanwhile, these anti-inflammatory effects of nicotine were abrogated by pre-treatment with AMPK inhibitor (compound C). These results suggested that the activation of AMPKα contributes to the anti-inflammatory effect of nicotine in vivo. Numerous studies have shown that the AMPK activator, 5-amino-4-imidazole carboxamide riboside (AICAR) could inhibit the NF-κB activation and vascular cell adhesion molecule (VCAM-1) expression induced by palmitate in cultured human umbilical vein endothelial cells [30]; inhibit the iNOS induction in cells and tissues exposed to inflammatory mediators [31]; and suppress LPS-induced proinflammatory mediators' production in RAW 264.7 cells and glial cells [32]. These findings are consistent with the role of AMPK in the inflammatory process reported in this study.

As shown in Fig. 2B and C, AMPKα was activated by nicotine in RAW 264.7 cells, and this effect was inhibited by pre-treatment with nACh receptor inhibitors, hexamethonium and α-bungarotoxin. These results suggested that the involvement of nACh receptor in nicotine-induced AMPKα phosphorylation in macrophages. Together with our data in vivo, our results corroborate the earlier reports that the anti-inflammatory effect of nicotine was mediated through α7 nACh receptor on macrophages [15,16].

The PI3K-Akt pathway has been shown to regulate negatively NF-κB and the expression of inflammatory genes [33]. Wortmannin, a specific inhibitor of PI3K, enhances LPS-induced nitric oxide production in murine peritoneal macrophages [33], and activation of PI3K-Akt suppresses LPS-induced lipoprotein lipase expression in J774 macrophages [34]. These results suggested that PI3K/Akt pathway plays an important role in the anti-inflammatory mechanism. Consistent with previous

suggestion, in the present study, nicotine was able to induce Akt phosphorylation, and this effect was abrogated by pre-treatment with hexamethonium and α-bungarotoxin (Fig. 3A). Moreover, wortmannin significantly blocked the activation of Akt and AMPKα induced by nicotine (Fig. 3B and C). These results suggest that nicotine promotes AMPKα phosphorylation in macrophages via sequential activation of the nACh receptor and PI3K/Akt pathway.

There appear to be at least two signaling pathways upstream of AMPKα, one triggered by an increase in the AMP/ATP ratio and dependent on LKB1 and one triggered by an increase in Ca^{2+} and dependent on CaMKK [8]. As shown in Fig. 4A, nicotine significantly increased CaMKK protein expression, but did not affect the expression of LKB1 protein (Fig. 4B). Base on this result, it suggested that LKB1 were unlikely to play an important role in the effects of nicotine. On the contrary, the expression of CaMKK induced by nicotine was abrogated by pre-treatment with hexamethonium and α-bungarotoxin. Furthermore, STO-609, a CaMKK inhibitor, inhibited the activation of AMPKα induced by nicotine (Fig. 4C). These data suggest that CaMKK contributed to nicotine-induced AMPK activation, nicotine promotes AMPKα phosphorylation in macrophages via sequential activation of the nACh receptor and CaMKK.

According to our data, it was worth to note that nicotine-induced AMPK phosphorylation may be mediated through PI3K/Akt pathway and CaMKK. It was recently reported that not only PDK1, but also CaMKK, can activate Akt via phosphorylation of Thr^{308}, which is located in the activation loop of Akt [35]. Our results confirm the downstream signaling role of Akt in CaMKK-mediated activation of AMPK induced by nicotine in macrophages on the basis of the following evidence. First, both of the CaMKK and Akt are involved in the nicotine-induced AMPK phosphorylation in macrophages (Figs. 3B, C and 4C). Second, nACh receptor mediated the effect of nicotine on the expression of CaMKK and the phosphorylation of Akt (Figs. 3A and 4A). Third, STO-609, a CaMKK inhibitor, abrogated the phosphorylation of Akt and AMPK induced by nicotine (Fig. 4C and D). These results also imply the interaction of PI3K/Akt pathway and CaMKK may exist and the detail interaction of PI3K/Akt and CaMKK needs to be evaluated in further studies.

In conclusion, AMPK is involved in the anti-inflammatory effect of nicotine, which is mediated through activation of the nACh receptor and the PI3K/Akt signaling pathway. CaMKK also contributes to the modulated PI3K/Akt pathway leading to affect the function of AMPK.

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