Effect of Nicotine on Advanced Glycation End Product-Induced Immune Response in Human Monocytes

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ABSTRACT

The up-regulation of adhesion molecule expressions on monocytes enhances cell-to-cell interactions with T cells, leading to cytokine production. Advanced glycation end products (AGEs) are modifications of proteins/lipids that become nonenzymatically glycated after contact with aldehyde sugars. Among various subtypes of AGEs, glyoxaldehyde-derived AGE (AGE-2) and glycoaldehyde-derived AGE (AGE-3) induce the expressions of intercellular adhesion molecule-1, B7.1, B7.2, and CD40 on monocytes, the production of interferon-γ and tumor necrosis factor-α, and the lymphocyte proliferation in human peripheral blood mononuclear cells. Nicotine is reported to inhibit the activation of monocytes via nicotinic acetylcholine receptor α7 subunit (α7-nAChR). In the present study, we found that nicotine inhibited the actions of AGE-2 and AGE-3. A nonselective and selective α7-nAChR antagonist, mecamylamine and α-bungarotoxin, reversed the inhibitory effects of nicotine, suggesting the involvement of α7-nAChR stimulation. Nicotine induced the expression of cyclooxygenase-2, prostaglandin E2 (PGE2), and cAMP in the presence and absence of AGE-2 and AGE-3. PGE2 is known to activate the EP2 receptor, increasing the cAMP level and protein kinase A (PKA) activity. The actions of nicotine were reversed in part by an EP2-receptor antagonist, AH6809, an EP2 receptor antagonist, AH23848, and a PKA inhibitor, N-[2-[4-bromocinnamyl(ami)n]ethyl]-5-isouquinolinesulfonamide dihydrochloride (H89). These results indicate that the mechanism of action of nicotine may be partially via endogenous PGE2 production.

Advanced glycation end products (AGEs), products of the nonenzymatic glycation/oxidation of proteins/lipids, accumulate during natural aging and are also greatly augmented in disorders, such as diabetes, renal failure, and Alzheimer’s disease (Schmidt et al., 1994; Brownlee, 1995; Takedo et al., 1996). Direct immunochemical evidence for the existence of four distinct AGE structures, including AGE-2, AGE-3, AGE-4, and AGE-5, has been identified within AGE-modified proteins and peptides (Takeuchi and Yamagishi, 2004). Among various subtypes of AGE, toxic AGE structures, AGE-2 and AGE-3, are the main structures of AGEs that are detectable in the serum of diabetic patients (Takeuchi and Yamagishi, 2004). AGE-2 and AGE-3 also have diverse biological activities on vascular wall cells, mesangial cells, Schwann cells, malignant melanoma cells, and cortical neurons (Okamoto et al., 2002; Yamagishi and Imaizumi, 2005). It is reported that AGEs ligate the cell-surface receptor for AGE (RAGE) on the vascular endothelium, monocytes, vascular smooth muscle, and neurons to activate cell-signaling pathways, such as p44/p42 mitogen-activated protein kinase and nuclear factor-κB (Yan et al., 1994; Lander et al., 1997), leading to the progression of pathogenesis of diabetic microvascular disease (Schmidt et al., 1994). It is noteworthy that AGEs up-regulate RAGE expression in various tissues, facilitating the AGE-RAGE response by forming a positive feedback pathways with AGEs. RAGE is known as the AGE-RAGE response by forming a positive feedback.
back loop (Yamagishi and Imaizumi, 2005). In a previous study, we found that AGE-2 and AGE-3 induced the expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α, and the lymphocyte proliferation in human PBMCs, but AGE-4 and AGE-5 had no effect (Takahashi et al., 2009a). The effect of AGE-2 and AGE-3 on the production of IFN-γ and TNF-α depended on cell-to-cell interaction via the engagement between ICAM-1, B7.1, B7.2, and CD40 on monocytes and their ligands on T cells, and the stimulation of RAGEs on monocytes was involved in the actions of AGE-2 and AGE-3 (Takahashi et al., 2009a; Wake et al., 2009).

Acetylcholine effectively deactivates peripheral macrophages and inhibits the release of proinflammatory mediators. Nicotine activates nAChR belonging to a family of ionotropic receptors consisting of five transmembrane subunits constituting ion channels. Non-neuronal cells such as monocytes and macrophages express nAChR (Wang et al., 2003, 2004). ACh-dependent macrophage deactivation is mediated by α7-nAChR, which is expressed in peripheral macrophages and has been described as being essential for the cholinergic anti-inflammatory pathway (Wang et al., 2003, 2004). On the other hand, a major product of COX-initiated arachidonic acid metabolism, PGE2, which is released from antigen-presenting cells, primes naive human T cells and enhances their production of anti-inflammatory cytokines while inhibiting their synthesis of pro-inflammatory cytokines (Coleman et al., 1994; Hempel et al., 1994). Among the four PGE2 receptor subtypes EP1, EP2, EP3, and EP4 receptors, activation of EP2 and EP4 receptors leads to an increase in cAMP levels and PKA activity (Bastien et al., 1994; Nataraj et al., 2001). In a previous study, we found that PGE2 inhibited AGE-2- and AGE-3-induced expressions of ICAM-1, B7.2, and CD40, the production of IFN-γ and TNF-α, and the lymphocyte proliferation via the EP2 and EP4 receptors (Takahashi et al., 2009b). The cAMP/PKA pathway was involved in the actions of PGE2-Nicotine is reported to induce the expressions of COX-2 and PGE2 in whole blood and microglia through α7-nAChR stimulation (Saareks et al., 1998; De Simone et al., 2005).

In the present study, we examined the effects of nicotine on AGE-2- and AGE-3-induced expressions of ICAM-1, B7.2, and CD40, the production of IFN-γ and TNF-α, and the lymphocyte proliferation in human PBMCs; we also investigated the involvement of PGE2 production in mediating these effects.

Materials and Methods

Reagents and Drugs. AGE-modified bovine serum albumin (BSA) (Sigma-Aldrich, St Louis, MO) was prepared as described previously (Takeuchi et al., 2000; Takahashi et al., 2009). In brief, each protein was incubated under sterile conditions with glyceraldehyde 3-phosphate (AGE-2) (Sigma-Aldrich) or glycolaldehyde (AGE-3) (Sigma-Aldrich) in 0.2 M phosphate buffer, pH 7.4, at 37°C for 7 days. AGE-BSA was dialyzed for 2 days at 4°C.

The endotoxin (AGE-3) (Sigma-Aldrich) in 0.2 M phosphate buffer, pH 7.4, at 37°C was purchased from Dako Denmark A/S (Glostrup, Denmark). FITC-conjugated mouse IgG1 mAb against CD54 and phycoerythrin-conjugated anti-CD4 mAb were purchased from Sigma-Aldrich. ACh-dependent macrophage deactivation is mediated by 7-nAChR, which is expressed in peripheral macrophages and has been described as being essential for the cholinergic anti-inflammatory pathway (Wang et al., 2003, 2004). On the other hand, a major product of COX-initiated arachidonic acid metabolism, PGE2, which is released from antigen-presenting cells, primes naive human T cells and enhances their production of anti-inflammatory cytokines while inhibiting their synthesis of pro-inflammatory cytokines (Coleman et al., 1994; Hempel et al., 1994). Among the four PGE2 receptor subtypes EP1, EP2, EP3, and EP4 receptors, activation of EP2 and EP4 receptors leads to an increase in cAMP levels and PKA activity (Bastien et al., 1994; Nataraj et al., 2001). In a previous study, we found that PGE2 inhibited AGE-2- and AGE-3-induced expressions of ICAM-1, B7.2, and CD40, the production of IFN-γ and TNF-α, and the lymphocyte proliferation via the EP2 and EP4 receptors (Takahashi et al., 2009b). The cAMP/PKA pathway was involved in the actions of PGE2-Nicotine is reported to induce the expressions of COX-2 and PGE2 in whole blood and microglia through α7-nAChR stimulation (Saareks et al., 1998; De Simone et al., 2005).

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Flow Cytometric Analysis for Adhesion Molecule Expression. Changes in the expressions of human leukocyte antigens, ICAM-1, B7.1, B7.2, CD40 and CD40L, on monocytes were examined by multicolor flow cytometry by use of a combination of anti-CD14 Ab with anti-ICAM-1, anti-B7.1, anti-B7.2, anti-CD40, or anti-CD40L Ab. PBMCs at 1 × 10⁶ cells/ml were incubated for 24 h. Cultured cells at 5 × 10⁵ cells/ml were prepared for flow cytometric analysis as described previously (Takahashi et al., 2003) and analyzed with a FACScan (BD Biosciences, San Jose, CA). The data were processed by use of the CellQuest program.

ELISA Assays. PBMCs at 1 × 10⁶ cells/ml were used to analyze IFN-γ and TNF-α production, and monocytes at 1 × 10⁶ cells/ml were used to analyze PGE2 production. After culturing for 24 h at 37°C in a 5% CO2/air mixture, cell-free supernatants were assayed for IFN-γ, TNF-α, and PGE2 protein (Cayman Chemical) by ELISA using the multiple Abs sandwich principle. The detection limits of ELISA for IFN-γ, TNF-α, and PGE2 were 10 pg/ml.

Proliferation Assay. PBMCs were divided into 96-well microplates, 200 μl/well, and were incubated with various conditions for 48 h, during which they were pulsed with 1 μCi of [3H]thymidine per well for the final 16 h. Then, the cells were harvested by the MicroMate 196 Cell Harvester (PerkinElmer Life and Analytical Sciences, Waltham, MA). Thymidine incorporation was measured by a β-counter (Matrix 9600; PerkinElmer Life and Analytical Sciences).

Western Immunoblotting. Monocytes at 1 × 10⁶ cells/ml were incubated with nicotine in the presence or absence of AGE-2 and AGE-3 at 37°C in a 5% CO2/air mixture for 30 min. After incubation, the cells were washed twice in phosphate-buffered saline before the addition of 60 μl of ice-cold lysis buffer (HEPES-buffered Hanks' balanced salt solution, pH 7.4, 0.5% Triton X-100, 10 mg/ml leupeptin, 10 mg/ml aprotinin) and 60 μl of sample buffer (0.125 M Tris base, pH 6.8, 20% glycerol, 4% SDS, 10% 2-mercaptoethanol). The samples were then heated at 95°C for 7 min before being stored at 20°C. Sample proteins (50 μl/lane) were separated on 9% acrylamide gel and transferred onto Trans-Blot membranes at 4°C for 16 h at 300 mA, after which the membranes were blocked for 1 h at 25°C in Tris-buffered saline (25 mM Tris-HCl, 0.2 M NaCl, 0.15% Tween 20, pH 7.6) containing 5% dried milk (wt/vol). Next, the membranes were incubated with trichloroacetic acid to a final concentration of 5% and 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase at 100 μM and frozen at −80°C. Frozen samples were subsequently sonicated and assayed for cAMP by use of a cAMP enzyme immunoassay kit (Cayman Chemical) according to the manufacturer’s instructions, for which no acetylation procedures were performed.

Statistical Analysis. Statistical significances were evaluated using ANOVA followed by Dunnett’s test. A probability value of less
than 0.05 was considered to indicate statistical significance. The results are expressed as the means ± S.E.M. of triplicate findings from five donors.

Results

Effects of Nicotine on AGE-2- and AGE-3-Induced Expression of ICAM-1, B7.1, B7.2, and CD40 on Monocytes, the Production of IFN-γ and TNF-α in PBMCs, and the Proliferation in PBMCs. In the previous study, to evaluate the binding of AGE subtypes to RAGE, we established an in vitro assay using immobilized AGE subspecies and the His-tagged soluble form of RAGE (sRAGE) protein (Takahashi et al., 2009a). AGE-2 and AGE-3 showed relatively high-affinity binding for sRAGE, whereas AGE-4 and AGE-5 showed moderate affinity for sRAGE. The appropriate incubation time and concentration of AGEs were determined according to the studies reported (Takahashi et al., 2009a; Wake et al., 2009). To determine appropriate incubation time, we examined the kinetics at 0, 4, 16, 24, and 48 h. In the absence of AGEs, the expression of ICAM-1, B7.1, B7.2, and CD40 moderately increased at 16 h and thereafter up to 24 and 48 h. AGE-2 and AGE-3 at 100 μg/ml significantly induced the expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α in PBMCs, and the proliferation in PBMCs at 16 h and thereafter up to 24 and 48 h, whereas AGE-4, AGE-5, and BSA at 100 μg/ml had no effect at all (Takahashi et al., 2009; Wake et al., 2009).

Moreover, to determine the appropriate concentration of AGEs, the effects of AGE-2 and AGE-3 at concentrations ranging from 100 ng/ml to 100 μg/ml for 24 h were examined. AGE-2 and AGE-3 at 10 and 100 μg/ml significantly induced the expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α in PBMCs, and the proliferation in PBMCs (Takahashi et al., 2009a; Wake et al., 2009).

As shown in Figs. 1 and 2, we established the effect of nicotine at concentrations ranging from 0.1 to 100 μM on 100 μg/ml AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α and the lymphocyte proliferation in PBMCs. Nicotine concentration-dependently inhibited AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2, and CD40 (Fig. 1, A and B), the production of IFN-γ and TNF-α, and the lymphocyte proliferation (Fig. 2, A and B). IC50 values for the inhibitory effect of nicotine on the expressions of ICAM-1, B7.1, B7.2, and CD40, the production of IFN-γ and TNF-α, and the lymphocyte proliferation in the presence of AGE-2 were 2, 1, 1, 2, 0.9, 1, and 0.9 μM, and those in the presence of AGE-3 were 1, 1, 2, 1, 0.9, 1, and 1 μM, respectively.

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Effects of α7-nAChR Antagonists on the Actions of Nicotine. To determine the involvement of α7-nAChR in nicotine activity, we examined the effect of a nonselective α7-nAChR antagonist, mecamylamine, and a selective α7-nAChR antagonist, α-bungarotoxin, ranging from 0.1 to 100 μM on 100 μM nicotine-induced inhibition of ICAM-1, B7.1,
B7.2, and CD40 expressions on monocytes (Fig. 3), the production of TNF-α/H9251 and IFN-γ/H9253, and the lymphocyte proliferation in PBMCs (Fig. 4) in the presence or absence of AGE-2 and AGE-3 at 100 g/ml. The 7-nAChR antagonists reversed the inhibitory effects of nicotine. Mecamylamine and -bungarotoxin had no effect in the absence of nicotine (data not shown).

Effects of Nicotine on Expression of COX-2 and Production of PGE2 in Monocytes. As shown in Fig. 5A, nicotine at 100 μM induced the expression of COX-2 in monocytes in the presence or absence of AGE-2 and AGE-3 at 100 μg/ml, but AGE-2 or AGE-3 alone had no effect. As shown in Fig. 5, B and C, nicotine induced PGE2 production in monocytes in the presence or absence of AGE-2 and AGE-3, but AGE-2 or AGE-3 alone had no effect. Mecamylamine (Fig. 5D) and -bungarotoxin (Fig. 5E) prevented nicotine-stimulated PGE2 production in the presence or absence of AGE-2 and AGE-3. Without nicotine, α7-nAChR antagonist inhibitors had no effect on PGE2 production (data not shown). A nonselective or a selective COX-2 inhibitor, indomethacin (Fig. 5F) or NS398 (Fig. 5G), inhibited nicotine-induced PGE2 production in the presence or absence of AGE-2 and AGE-3, but a PKA inhibitor, H89, had no effect (data not shown). Without nicotine, these inhibitors had no effect on PGE2 production (data not shown).

Fig. 2. The effects of nicotine on AGE-2-, AGE-3-, and BSA-induced production of IFN-γ and TNF-α and the lymphocyte proliferation in PBMCs. PBMCs at 1 × 10⁶ cells/ml were incubated with AGE-2 (A), AGE-3 (B), and BSA (C) at 100 μg/ml and nicotine at increasing concentrations from 0.1 to 100 μM for 24 h. IFN-γ and TNF-α concentrations in conditioned media were determined by ELISA. The lymphocyte proliferation was determined by [³H]thymidine uptake as described under Materials and Methods. A–C, filled circles represent the effect of nicotine on adhesion molecule expression and cytokine production in the presence of AGE-2 and AGE-3. A, open circles represent the effect of nicotine in the absence of AGE-2 and AGE-3. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. *, P < 0.05; ***, P < 0.01 compared with the value for AGE-2 and AGE-3. When an error bar is within a symbol, the bar was omitted.

Fig. 3. The effect of α7-nAChR antagonists on the effect of nicotine on the expressions of ICAM-1, B7.1, B7.2, and CD40. The effect of a nonselective α7-nAChR antagonist, mecamylamine (○), or a selective α7-nAChR antagonist, -bungarotoxin (●), at increasing concentrations ranging from 0.01 to 10 nM on the actions of nicotine at 100 μM in the presence of AGE-2 (A) and AGE-3 (B) at 100 μg/ml. The expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes were determined by flow cytometry. The results are expressed as the means ± S.E.M. of triplicate findings from five donors. *, P < 0.05; ***, P < 0.01 compared with the value for AGE-2 and AGE-3. When an error bar is within a symbol, the bar was omitted.

Fig. 4. The effect of α7-nAChR antagonists on the effect of nicotine on the production of IFN-γ and TNF-α and the lymphocyte proliferation. The effect of a nonselective α7-nAChR antagonist, mecamylamine (●), or a selective α7-nAChR antagonist, -bungarotoxin (○), at increasing concentrations ranging from 0.01 to 10 nM on the actions of nicotine at 100 μM in the presence of AGE-2 (A) and AGE-3 (B) at 100 μg/ml. IFN-γ and TNF-α concentrations in conditioned media were determined by ELISA. The lymphocyte proliferation was determined by [³H]thymidine uptake as described in Materials and Methods. The results are expressed as the means ± S.E.M. of triplicate findings from five donors. *, P < 0.05; ***, P < 0.01 compared with the value for AGE-2 and AGE-3. When an error bar is within a symbol, the bar was omitted.
Effects of Nicotine on the Activation of cAMP in Monocytes. In the presence or absence of AGE-2, AGE-3, and BSA at 100 μg/ml, nicotine at 100 μM significantly activated intracellular cAMP in monocytes at 15 min and thereafter up to 30 and 60 min (Fig. 6A). As shown in Fig. 6B, 7-nAChR antagonists, mecamylamine and α-bungarotoxin, prevented nicotine-induced activation of cAMP in the presence or absence of AGE-2, AGE-3, and BSA at 100 μg/ml for the indicated periods. C, the effect of nicotine at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2, AGE-3, and BSA at 100 μg/ml for 24 h was determined. D-G, the effect of a nonselective α7-nAChR antagonist, mecamylamine (D); a selective α7-nAChR antagonist, α-bungarotoxin (E); a nonselective COX-2 inhibitor, indomethacin (F); and a selective COX-2 inhibitor, NS398 (G), on the actions of nicotine at 100 μM was determined. Filled circles represent the effect of nicotine on PGE2 production in the presence of AGE-2, open squares represent that effect in the presence of AGE-3, and open squares represent that effect in the presence of BSA. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. When an error bar is within a symbol, the bar is omitted.

Involvement of Prostanoid EP2 and EP4 Receptors in the Actions of Nicotine. To determine the involvement of PGE2 receptor subtypes in the effects of nicotine on the expressions of ICAM-1, B7.1, B7.2, and CD40, the production of IFN-γ and TNF-α and the lymphocyte proliferation, the effect of an EP2 receptor antagonist, AH6809 (Kay et al., 2006; Takahashi et al., 2009b) and an EP4-receptor antagonist, AH23848 (Kay et al., 2006; Takahashi et al., 2009b) at concentrations ranging from 0.1 to 100 μM in the presence of nicotine at 100 μM were examined (Figs. 7 and 8). AH6809 and AH23848 reversed the inhibitory effect of nicotine on AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2, and CD40 (Fig. 7), the production of IFN-γ and TNF-α, and the lymphocyte proliferation (Fig. 8) in a concentration-dependent manner. The effect of AH6809 on the actions of nicotine was stronger than that of AH23848. On the other hand, AH6809 and AH23848 had no effect on the actions of AGE-2 and AGE-3 in the absence of nicotine.

Involvement of COX-2 and PKA in the Action of Nicotine. To investigate the involvement of COX-2 and PKA in the actions of nicotine, the effects of COX-2 inhibitors, indomethacin and NS398, on the actions of nicotine were determined (Fig. 9). Indomethacin and NS398 reversed the inhibitory effect of nicotine on AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2, and CD40 (Fig. 9A), the production of IFN-γ and TNF-α, and the lymphocyte proliferation (Fig. 9B) in a concentration-dependent manner. The effect of indomethacin on the actions of nicotine was stronger than that of NS398. On the other hand, indomethacin and NS398 had no effect on the actions of AGE-2 and AGE-3 in the absence of nicotine.
the inhibitory effect of nicotine on the expressions of ICAM-1, B7.1, B7.2, and CD40, the production of IFN-γ and TNF-α, and the lymphocyte proliferation, we examined the effect of COX-2 and PKA inhibitors (Figs. 9 and 10). COX-2 inhibitors, indomethacin and NS398, and the PKA inhibitor, H89, reversed the inhibitory effect of nicotine on the expressions of adhesion molecule (Fig. 9), the production of IFN-γ and TNF-α, and the lymphocyte proliferation (Fig. 10) in the presence of AGE-2 and AGE-3. COX-2 and PKA inhibitors had no effect on AGE-2- and AGE-3-induced adhesion molecule expression and cytokine production in the absence of nicotine (data not shown).

Discussion

The level of AGE-2 is reported to be 17 μg/ml in the serum of patient with diabetes (Enomoto et al., 2006; Nakamura et al., 2007). It is reported that AGEs at the concentrations ranging from 50 to 200 μg/ml remarkably induce human monocyte adhesion to bovine retinal endothelial cells (Mamputu and Renier, 2004). AGEs at 200 μg/ml induce the expression of CD40, CD80 and CD86 and the production of IFN-γ in dendritic cells (Ge et al., 2005). In the previous study, we found that AGE-2 and AGE-3 at 10 and 100 μg/ml significantly up-regulated the expressions of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and TNF-α and the lymphocyte proliferation (Takahashi et al., 2009a; Wake et al., 2009). Therefore, the concentration (100 μg/ml) used in the present study may be not far above the pathological concentration of AGEs in the serum of patient with diabetes reported in the studies (Enomoto et al., 2006; Nakamura et al., 2007).

We found that AGE-2 and AGE-3 induced the production of IFN-γ and TNF-α in monocytes isolated from PBMCs, exhibiting 20% of the amount obtained in PBMCs (Takahashi et al., 2009a). AGE-2 and AGE-3 had no effect on the production of IFN-γ and TNF-α in T cells isolated from PBMCs. Anti-ICAM-1, anti-B7.1, anti-B7.2, and anti-CD40 Abs inhibited the AGE-2- and AGE-3-induced production of IFN-γ and TNF-α in PBMCs. We suggested that AGE-2- and AGE-3-induced cytokine production required the enhancement of cell-to-cell interaction between monocytes and T cells through the induction of plural adhesion molecule expression on monocytes. Together with these results, we examined the effect of nicotine on AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α in PBMCs, and the lymphocyte proliferation in PBMCs.

As shown in Figs. 1 and 2, we found that nicotine prevented 100 μg/ml AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α, and the lymphocyte proliferation in PBMCs. In the presence of AGE-2 and AGE-3 at 10 μg/ml, nicotine also inhibited expressions of ICAM-1, B7.1, B7.2, and CD40, the produc-

Fig. 7. The effects of prostanoid receptor antagonists on the inhibitory effect of nicotine on the production of IFN-γ and TNF-α and the lymphocyte proliferation. PBMCs at 1 × 10⁶ cells/ml treated with nicotine at 100 μM were incubated with the EP2-receptor antagonist AH6809 (A) and the EP4-receptor antagonist AH23848 (B) at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2 and AGE-3 at 100 μg/ml. The expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes were determined by flow cytometry. Filled circles represent the effect of antagonists on nicotine-inhibited adhesion molecule expression in the presence of AGE-2. Open circles represent the effect of antagonists on nicotine-inhibited adhesion molecule expression in the presence of AGE-3. Open squares represent the effect of antagonists on the actions of AGE-2 in the absence of nicotine. Open squares represent the effect of antagonists on the actions of AGE-2 in the absence of nicotine. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ∗, P < 0.05; ††, P < 0.01 compared with the value for nicotine in the presence of AGE-2 and AGE-3. When an error bar is within a symbol, the bar was omitted.

Fig. 8. The effects of prostanoid receptor antagonists on the inhibitory effect of nicotine on the production of IFN-γ and TNF-α and the lymphocyte proliferation. PBMCs at 1 × 10⁶ cells/ml treated with nicotine at 100 μM were incubated with the EP2-receptor antagonist AH6809 (A) and the EP4-receptor antagonist AH23848 (B) at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2 and AGE-3 at 100 μg/ml. IFN-γ and TNF-α concentrations in conditioned media were determined by ELISA. The lymphocyte proliferation was determined by [3H]thymidine uptake as described under Materials and Methods. Filled circles represent the effect of antagonists on nicotine-inhibited adhesion molecule expression in the presence of AGE-2. Open circles represent the effect of antagonists on nicotine-inhibited adhesion molecule expression in the presence of AGE-3. Open squares represent the effect of antagonists on the actions of AGE-2 in the absence of nicotine. Open squares represent the effect of antagonists on the actions of AGE-3 in the absence of nicotine. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ∗, P < 0.05; ††, P < 0.01 compared with the value for nicotine in the presence of AGE-2 and AGE-3. When an error bar is within a symbol, the bar was omitted.
tion of IFN-γ and TNF-α, and the lymphocyte proliferation in a concentration-dependent manner (data not shown). The IC50 values of nicotine for the inhibition of adhesion molecule and cytokine production were within the range of the concentration reported to be effective in recent animal studies (Wang et al., 2003, 2004). It is suggested that vagus nerve stimulation modulates the immune response and controls inflammation through a nicotinic anti-inflammatory pathway dependent on 7-nAChR (de Jonge et al., 2005; Saeed et al., 2005). 7-nAChR is required for acetylcholine inhibition of lipopolysaccharide-induced TNF-α production in human macrophages (Wang et al., 2003). In the present study, we found that 7-nAChR antagonists reversed the inhibitory effect of nicotine on the expressions of ICAM-1, B7.1, B7.2, and CD40, the production of IFN-γ and TNF-α and the lymphocyte proliferation in the presence of AGE-2 and AGE-3 (Fig. 3 and 4). This suggests that inhibitory effects of nicotine depend on the stimulation of 7-nAChR.

We further investigated the action mechanism of nicotine. PGE2, which is released from antigen-presenting cells, acts on naive human T cells to enhance their production of anti-inflammatory cytokines (Hempel et al., 1994; Coleman et al., 1994). PGE2, a product of COX-2-initiated metabolism, is known to activate the cAMP/PKA pathway (Bastien et al., 1994; Nataraj et al., 2001). As shown in Fig. 5A and B, we found that nicotine induced the expression of COX-2 and the production of PGE2 in the presence or absence of AGE-2 and AGE-3. The α7-nAChR and COX-2 inhibitors prevented nicotine-initiated PGE2 production in the presence or absence of AGE-2 and AGE-3 (Fig. 5C–F). These results suggest that the effect of nicotine on the expression of COX-2 and the production of PGE2 depends on the stimulation of α7-nAChR.

We also determined the levels of other COX-2 metabolites, including PGE1, PGD2, PGF2, PGI2, PGJ2, and thromboxane, measured in monocyte medium treated with nicotine in the presence or absence of AGE-2 and AGE-3, but all were under the level of detection (data not shown). As shown in Fig. 6A, nicotine activated intracellular cAMP in monocytes in the presence or absence of AGE-2 and AGE-3 (Fig. 6B), suggesting the involvement of α7-nAChR stimulation and COX-2 production in nicotine-induced elevation of cAMP.

In the previous study, we reported that PGE2 inhibited AGE-2- and AGE-3-enhanced expressions of ICAM-1, B7.1, B7.2, and CD40 on monocytes, the production of IFN-γ and TNF-α, and the lymphocyte proliferation in PBMCs (Taka-
hashi et al., 2009b). Among the four subtypes of receptors: prostanoid EP1, EP2, EP3, and EP4 receptors, the EP2 and EP4 receptors were involved in the actions of PGE2. In the previous study, we found that the EP2-receptor antagonist, AH6809 and the EP4-receptor antagonist, AH23848, inhibited the actions of PGE2 (Takahashi et al., 2009b). An EP3-receptor agonist, ONO-ÆE1-259-01 and an EP2-receptor agonist, ONO-ÆE1-329 mimicked the actions of PGE2. In the presence of AGE-2 and AGE-3, PGE2 induced the elevation of cAMP via EP2 and EP4 receptor. Moreover, the PKA inhibitor, H89, inhibited the action of PGE2, and a membrane-permeable cAMP analog, dibutyryl cAMP, and an adenylate cyclase activator, forskolin, mimicked the effect of PGE2. These results suggested the involvement of the EP2/EP4 receptors-cAMP/PKA pathway in the actions of PGE2.

In the present study, we found that the EP2-receptor antagonist, AH6809 and the EP4-receptor antagonist, AH23848, inhibited the actions of nicotine (Figs. 7 and 8). As shown in Figs. 9 and 10, COX-2 or PKA inhibitors partially reversed the inhibitory effect of nicotine on the expressions of ICAM-1, B7.1, 9 and 10, COX-2 or PKA inhibitors partially reversed the inhibited actions of nicotine (Figs. 7 and 8). As shown in Figs. 7 and 8, the stimulation of SMCs and endothelial cells, monocytes, macrophages, mesangial cells, and nerve cells (Brett et al., 1993). AGEs directly stimulate proliferation not in endothelial cells, but in SMCs (Satoh et al., 1997). It is reported that PGE2 induced by monocytes inhibits procollagen secretion by human vascular SMCs, leading to extracellular matrix remodeling and resistance to rupture during atherosclerosis (Fitzsimmons et al., 1999). Elevation of cAMP in endothelial cells inhibits proliferation, leading to the inhibition of atherosclerosis in patients with diabetes (Lorenzwicz et al., 2007). Nicotine exerts its atherogenic effects in part through the increase of SMC proliferation and migration (Carty et al., 1997; Di Luozzo et al., 2005). α7-nAChR is detected in the rat arterial system and in SMCs derived from brain basilar arteries (Brüggmann et al., 2003; Li et al., 2004), indicating that stimulation of α7-nAChR induces the proliferation of SMCs. Together with these results and our data, other extracellular stimuli, which induce intracellular PGE2 production upon binding to their cognate G protein-coupled receptors, may regulate the activation of vascular smooth muscle cells and endothelial cells. However, α7-nAChR stimulation may inhibit the activation of monocytes. Further study of the role of α7-nAChR in the stimulation of SMCs and monocytes should be continued.

In conclusion, we found that nicotine inhibited AGE-2- and AGE-3-enhanced adhesion molecule expressions, the cytokine production, and the lymphocyte proliferation via α7-nAChR. The COX-2-PGE2-cAMP/PKA system may be involved, in part, in the actions of nicotine. Through the inhibition of toxic AGE-dependent responses in monocytes, the stimulation of α7-nAChR may partially contribute to regulation of the development of atherosclerotic plaques in diabetes.

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References


Effect of Nicotine on AGEs-Treated Monocytes


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