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Effects of nicotine on K^+ channel currents in vascular smooth muscle cells from rat tail arteries

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Abstract

Intake of nicotine has been related in many cases to acute or chronic hypertension. Using the patch–clamp technique the effect of nicotine on voltage-dependent K^+ channel currents in rat tail artery smooth muscle cells was studied. Nicotine at concentrations of 1–100 μM or 0.3–3 mM increased or decreased, respectively, the amplitude of the tetraethylammonium-sensitive K^+ currents. Pretreatment of cells with 10 μM dihydro-β-erythroidine hydrobromide, a nicotinic receptor antagonist, abolished the excitatory effect ($n = 6$), but not the inhibitory effect ($n = 10$), of nicotine on K^+ channel currents. The activation of nicotinic receptors with 100 μM 1,1-dimethyl-4-phenylpiperazinium iodide increased K^+ channel currents by $27.4 \pm 3.8\%$ ($n = 13$, $P < 0.01$). Our results indicate that the excitatory and inhibitory effects of nicotine on K^+ channels are respectively mediated by a nicotinic receptor-dependent mechanism and by a direct interaction of nicotine with K^+ channels.

Keywords: Nicotine; Patch–clamp; Peripheral artery; Smokeless tobacco; Tetraethylammonium

1. Introduction

Cigarette smoking is known to be one of the risking factors for hypertension and stroke. Consumption of smokeless tobacco also caused acute hypertension in humans (Nowak, 1994; Westman, 1995). The most important substance in cigarette smoke or smokeless tobacco to disturb cardiovascular functions is nicotine, a tertiary amine composed of a pyridine and a pyrrolidine ring. Nicotine in smokeless tobacco is absorbed rapidly through the oral or nasal mucosa (Russell et al., 1981). Absorbed nicotine may release catecholamine from sympathetic nerve endings. The activation of α adrenoceptors in vascular smooth muscle cells then contracts vascular tissues and elevates blood pressure (Paulson et al., 1994; Toda et al., 1995). On the other hand, the activation of β adrenoceptors in vascular smooth muscle cells leads to the relaxation of vascular tissues (Toda et al., 1995). Nicotine may also directly act on vascular smooth muscle cells to induce vascular relaxation or contraction but these direct vascular actions of nicotine have not been intensively studied. Furthermore, little information is available on whether ion channel functions in vascular smooth muscle cells are affected by nicotine either via the activation of nicotinic receptors or through a direct effect on nicotine-sensitive ion channels.

In the present study, the patch–clamp technique was applied on single vascular smooth muscle cells from rat tail arteries. The time-dependent and concentration-dependent effects of nicotine on voltage-dependent K^+ channel currents were investigated. The role of nicotinic receptor activation in the effects of nicotine on K^+ channel currents was examined. Characterization of the nicotine-induced modulations of K^+ channels in vascular smooth muscle cells as well as the underlying mechanisms will help to better understand the ionic mechanisms of the complex vascular effects of nicotine.

2. Materials and methods

2.1. Cell preparation

Single smooth muscle cells were isolated and identified as previously described (Wang et al., 1989). Male Sprague–Dawley rats (150–200 g) were anesthetized by

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intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight). After isolation of tail arteries the rats were sacrificed by overdose injection of pentobarbital. The isolated tail arteries were cut open longitudinally, and enzymatically digested with collagenase/dispase, elastase and collagenase for different periods of time. The tissues were then triturated and isolated cells were plated in 35 mm Petri dishes and cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in a CO_2 incubator at 37°C. The cells were used 8–36 h after isolation unless otherwise indicated. Following these procedures, almost all the harvested cells were smooth muscle cells with elongated shape and intact contractility (Wang et al., 1989, 1995). Experimental protocols were approved by the University Committee on Animal Care and Supply of the University of Saskatchewan.

2.2. Whole-cell recording of K^+ channel currents

Briefly, the Petri dish with attached cells was mounted on the stage of an inverted phase contrast microscope. Pipettes with tip resistances of 2–8 MΩ were used. Membrane currents in smooth muscle cells were recorded using an Axopatch-200A patch–clamp amplifier, controlled by a Digidata 1200 interface and a pClamp software (version 6.02, Axon Instruments). Membrane currents were filtered at 1 kHz with a 4-pole Bessel filter, digitized and stored. At the beginning of each experiment, junctional potential between pipette solution and bath solution was electronically adjusted to zero (Wu et al., 1996; Wang et al., 1997; Wu et al., 1997). The bath solution contained (mM): NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.2, HEPES 10, and glucose 10. The patch pipettes were filled with (mM): KCl 140, Na_2ATP 5, HEPES 10, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 11, GTP 1. Test pulses were made with a 10 mV increment from −100 to +60 mV. The holding potential was set at −70 mV at which voltage-dependent K^+ channels were not inactivated. *I–V* curves were constructed using the sustained current amplitude at the end of 800 ms test pulses. The osmolality and pH of all pipette and bath solutions were adjusted to 290 mOsm/l and 7.40, respectively. Unless otherwise indicated, cells were continuously superfused with the bath solution containing tested chemicals at desired final concentrations.

2.3. Chemicals and data analysis

Nicotine, 1,1-dimethyl-4-phenylpiperazinium iodide, dihydro-β-erythroidine hydrobromide and other chemicals were purchased from Sigma (St. Louis, USA). Data were expressed as means ± S.E.M. Data were analyzed using Student's *t*-test, analysis of variance in conjunction with the Newman–Keuls test and analysis of variance for repeated measures (MANOVA) where applicable. Group differences were considered statistically significant at the level of $P < 0.05$.

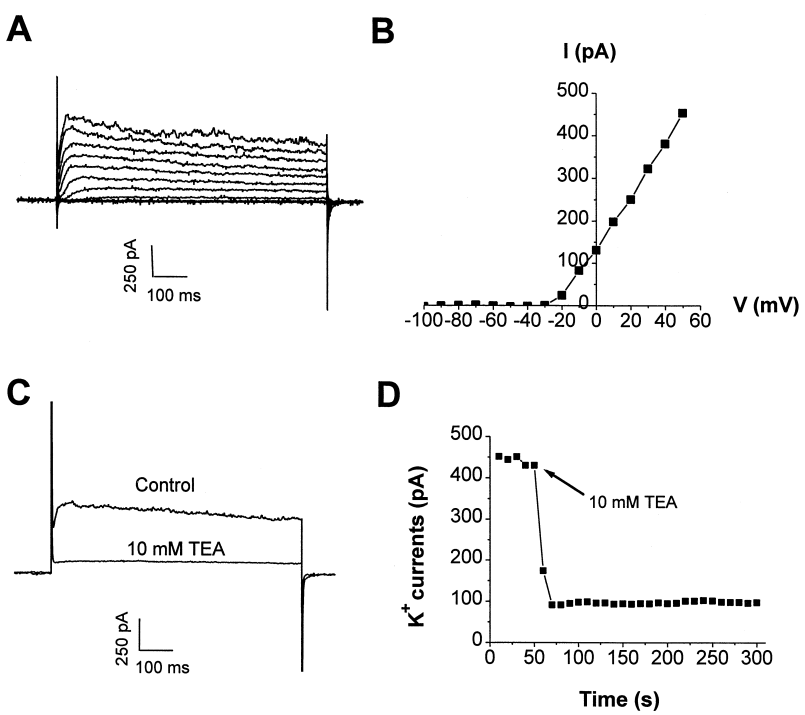

Fig. 1. Voltage-dependent K^+ channel currents in isolated rat tail artery smooth muscle cells. The actual current traces and *I–V* relationship of K^+ channels in the same cell were shown in (A) and (B). The K^+ channel currents were significantly inhibited by tetraethylammonium (TEA, 10 mM) in another cell as shown in (C) (test potential, +30 mV). (D) shows the time course of the inhibitory effect of tetraethylammonium in the same cell as in (C) (test potential, +30 mV).

3. Results

K^+ channel currents in rat tail artery smooth muscle cells were characterized by their delayed activation, relative lack of inactivation during 800 ms depolarization pulses, and outward rectification (Fig. 1). The application of tetraethylammonium to the cell immediately blocked K^+ currents (Fig. 1D) in a reversible (Fig. 2A) and concentration-dependent manner (Fig. 2B). However, 4-aminopyridine, the antagonist of the fast inactivated K^+ channels (I_A channel), at 1 mM had no effect on K^+ currents (Fig. 2B). The delayed rectifier K^+ currents are inhibited by 4-aminopyridine in some types of vascular smooth muscle cells, but obviously not sensitive to 4-aminopyridine in rat tail artery smooth muscle cells. Nicotine at concentrations of 1–100 μM increased the amplitude of the outward K^+ channel currents in rat tail artery smooth muscle cells (Fig. 3). The maximum excitatory effect of nicotine was achieved approximately 3–5 min after application (Fig. 3B). At 100 μM, nicotine increased K^+ channel currents from 206.1 ± 10.9 pA to 242.2 ± 9.5 pA (holding potential, −70 mV; test potential, +30 mV. $n = 20$, $P < 0.01$). K^+ channel currents were inhibited by nicotine at concentrations of 300 μM to 3 mM (Fig. 4). At 1 mM, nicotine inhibited K^+ currents from 204 ± 13 pA to 95 ± 6 pA ($n = 21$, $P < 0.01$). The inhibitory effect of nicotine on K^+ channel currents occurred instantly and lasted for at least 5 min in the presence of nicotine (Fig. 4B). Upon washing out nicotine from the bath solution, the inhibitory effect on K^+ channel currents was reversed (Fig. 4C). The I–V relationships of K^+ channel currents in the absence and then presence of nicotine revealed that at membrane potentials from −60 to 0 mV nicotine had no effects on K^+ channel currents (Fig. 3C and Fig. 4C).

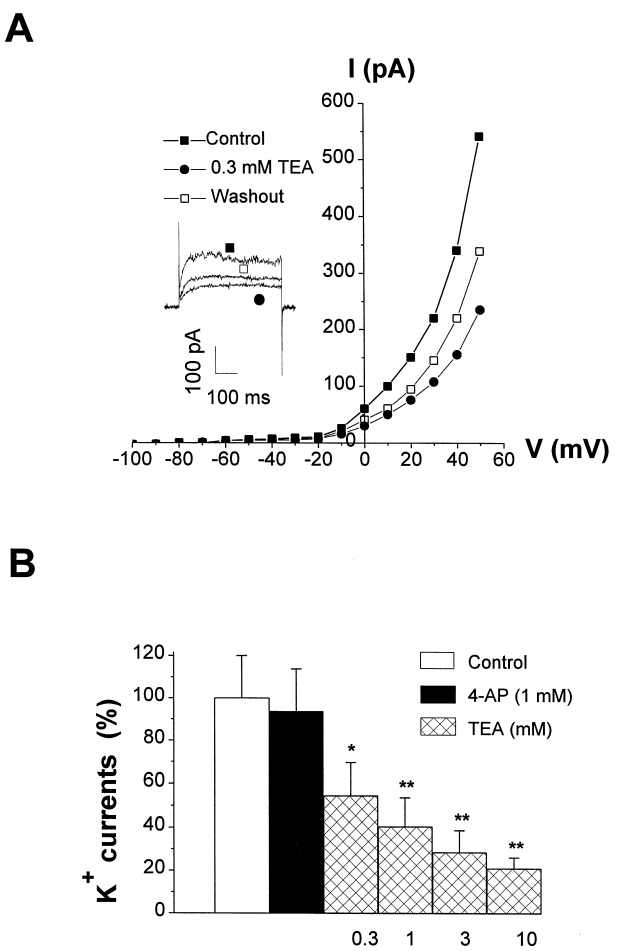

Fig. 2. The sensitivities of K^+ channel currents to tetraethylammonium (TEA) and 4-aminopyridine (4-AP) in rat tail artery smooth muscle cells. (A) The I–V relationships of K^+ channel currents in the absence and then presence of tetraethylammonium. Representative current traces were shown in the inset (test potential, +30 mV). (B) The effects of tetraethylammonium and 4-aminopyridine on K^+ channel currents. Test potential, +30 mV. $n = 5$–8 for each group. $*$ $P < 0.05$ vs. control; $**$ $P < 0.01$ vs. control.

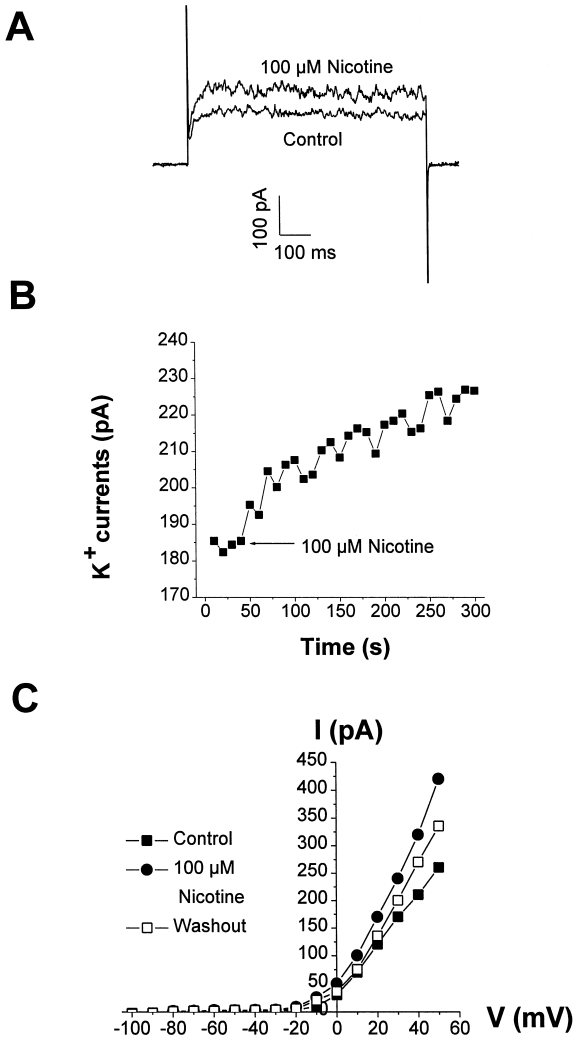

Fig. 3. The excitatory effect of nicotine on K^+ channel currents in rat tail artery smooth muscle cells. (A) Representative current traces before and after the application of nicotine (100 μM). Test potential, +30 mV. (B) The time course of the excitatory effect of nicotine on K^+ channel currents. Holding potential, −70 mV; test potential, +30 mV. (C) The I–V relationships of K^+ channel currents in the absence and then presence of nicotine (100 μM).

The reproducible and concentration-dependent effects of nicotine, both excitatory and inhibitory, on K^+ channel currents are summarized in Fig. 5.

To examine whether the nicotine-induced modulation of K^+ channel currents in vascular smooth muscle cells was mediated by nicotinic receptors, the effects of nicotinic receptor antagonist and agonist were further studied. Nicotinic receptor antagonist dihydro-β-erythroidine hydrobromide at 100 pM ($n = 3$), 0.5 μM ($n = 10$), or 10 μM ($n = 10$) had no direct effect on K^+ channel currents (not shown). The inhibitory effect of nicotine (1 mM) on K^+ channel currents was not affected by pretreatment of cells with dihydro-β-erythroidine hydrobromide at 0.5 μM ($n = 5$) (not shown) or 10 μM ($n = 10$) (Fig. 6). In the presence of 10 μM dihydro-β-erythroidine hydrobromide, 1 mM nicotine inhibited K^+ currents from 199 ± 10 pA to 86 ± 4 pA ($n = 10$, $P > 0.05$ vs. the effect of 1 mM nicotine in the absence of dihydro-β-erythroidine hydrobromide). In contrast, the excitatory effect of nicotine (100 μM) on K^+ channel currents was inhibited by 10 μM dihydro-β-erythroidine hydrobromide ($n = 10$) (Fig. 7A). Moreover, nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium iodide (100 μM) alone increased K^+ channel currents from 198 ± 14 pA to 252 ± 16 pA ($n = 13$, $P < 0.01$) (Figs. 7 and 8). As summarized in Fig. 8, the excitatory effect of nicotine at 100 μM on K^+ channel currents in smooth muscle cells were abolished by pretreatment of the cells with 10 μM dihydro-β-erythroidine hydrobromide for 5–6 min ($n = 10$, $P > 0.05$).

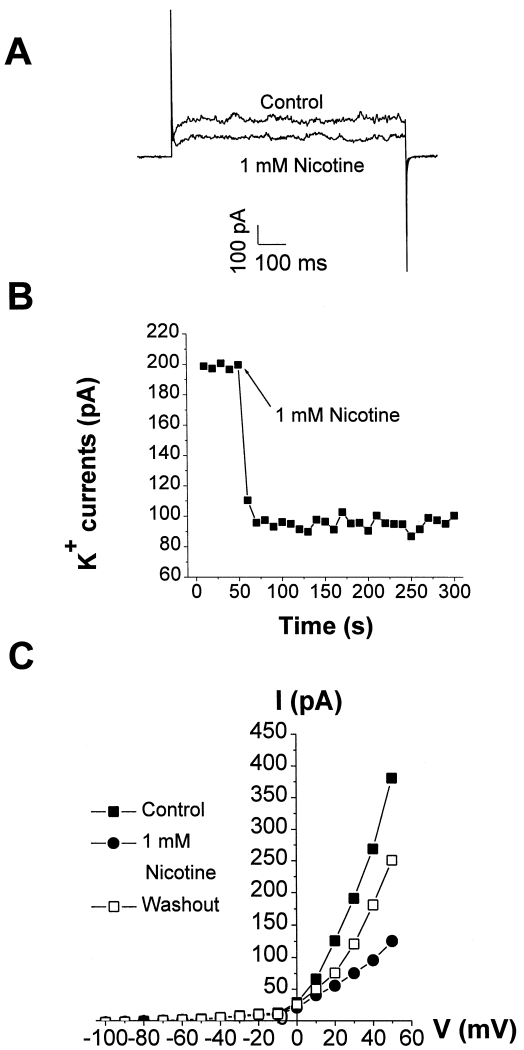

Fig. 4. The inhibitory effect of nicotine on K^+ channel currents in rat tail artery smooth muscle cells. (A) Representative current traces before and after the application of nicotine (1 mM). Test potential, +30 mV. (B) The time course of the inhibitory effect of nicotine on K^+ channel currents. Holding potential, −70 mV; test potential, +30 mV (C). The $I–V$ relationships of K^+ channel currents in the absence and then presence of nicotine (1 mM).

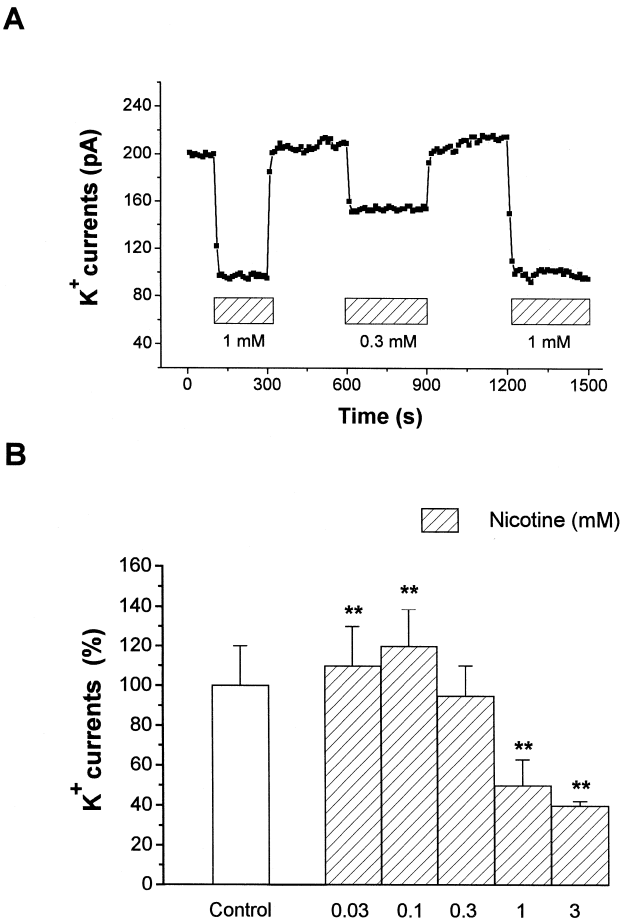

Fig. 5. Summary of the effects of nicotine on K^+ channel currents in rat tail artery smooth muscle cells. Test potential, +30 mV. (A) The reproducibility of the inhibitory effects of nicotine at difference concentrations on K^+ channel currents. (B) The concentration-dependent excitatory and inhibitory effects of nicotine on K^+ channel currents. $n = 5–9$ for each group. $* P < 0.05$ vs. control; $** P < 0.01$ vs. control.

4. Discussion

It is known that the blood levels of nicotine fall rapidly after cigarette smoking. In contrast, the blood levels of nicotine sustain during and after smokeless tobacco use synchronized with the continuous absorption of nicotine

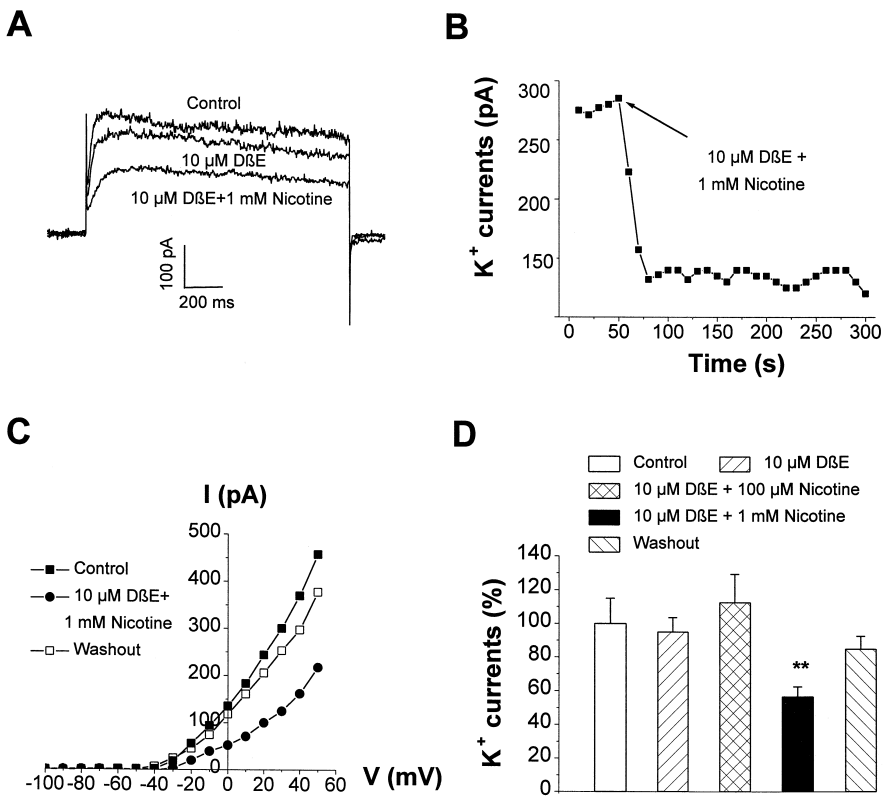

Fig. 6. The influence of dihydro-β-erythroidine (DβE) on the effects of nicotine on K^+ channel currents in rat tail artery smooth muscle cells. (A) The presence of dihydro-β-erythroidine hydrobromide (10 μM) did not alter the inhibitory effect of nicotine (1 mM) on K^+ channel currents (test potential, +30 mV). (B) The time course of the inhibitory effect of nicotine on K^+ channel currents in the presence of dihydro-β-erythroidine hydrobromide (test potential, +30 mV). (C) *I–V* relationships of K^+ channel currents under different experimental conditions. (D) Although dihydro-β-erythroidine hydrobromide (10 μM) had no effect on the inhibitory effect of nicotine (1 mM) on K^+ channel currents, it inhibited the excitatory effect of nicotine (100 μM). Test potential, +30 mV. $**P < 0.05$. $n = 5–10$ for each group.

even after smokeless tobacco use (Benowitz, 1988). In comparison with smoking an average strength cigarette, the typical single dose of nicotine in chewing tobacco that will be absorbed is over 15 times greater (Spangler and Salisbury, 1995; Westman, 1995). Benowitz et al. (1990) reported that nicotine content in single dose of cigarette or chewing tobacco could reach 8.4 mg or 133 mg, respectively. This amount of nicotine dissolved in 5 l of average blood volume of a normal adult (Guyton, 1986) represents the blood levels of nicotine of approximately 10 to 164 μM. These estimated levels could be much higher in certain smokeless tobacco users considering the significant individual variance among smokeless tobacco users, an 8-fold range in peak plasma nicotine concentration (Benowitz, 1993). Furthermore, the peak value and the accumulated level of nicotine could be much higher than stabilized single dose levels. With an average half-life of 2 to 3 h, nicotine accumulates for 6 to 8 h (Benowitz et al., 1989) with a daily intake of more than 1000 mg nicotine for regular chewing tobacco use (Benowitz et al., 1990). In our studies, the effects of nicotine on K^+ channel currents can be observed at concentrations greater than 10 μM. Therefore, the concentrations of nicotine tested in our study are closely related to the blood concentrations of nicotine in smokeless tobacco users.

The effects of nicotine on ion channel currents have only been studied in few cases. Using the whole-cell configuration of the patch–clamp technique, Hamon et al. (1997) found that nicotine increased reversibly the firing rate of cultured rat striatal neurons and depolarized cell membrane. This effect was related to the inhibition of voltage-dependent K^+ channels by nicotine (10 μM) and nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide (100 μM). It was further hypothesized that nicotine affected K^+ channels via the stimulation of nicotinic receptors since dihydro-β-erythroidine hydrobromide (10 μM) suppressed the inhibitory effect of nicotine on K^+ channel currents. An increase in K^+ channel currents by nicotine, on the other hand, has also been suggested in one study. Marks et al. (1995) reported that 4-aminopyridine and tetraethylammonium, two blockers of delayed outward rectifier K^+ channels, completely inhibited nicotine-stimulated $^{86}Rb^+$ efflux in mouse brain synaptosomes. These results can be interpreted as the competitive blockade of nicotinic receptors by 4-aminopyridine and tetraethylammonium (Marks et al., 1995) or as the direct

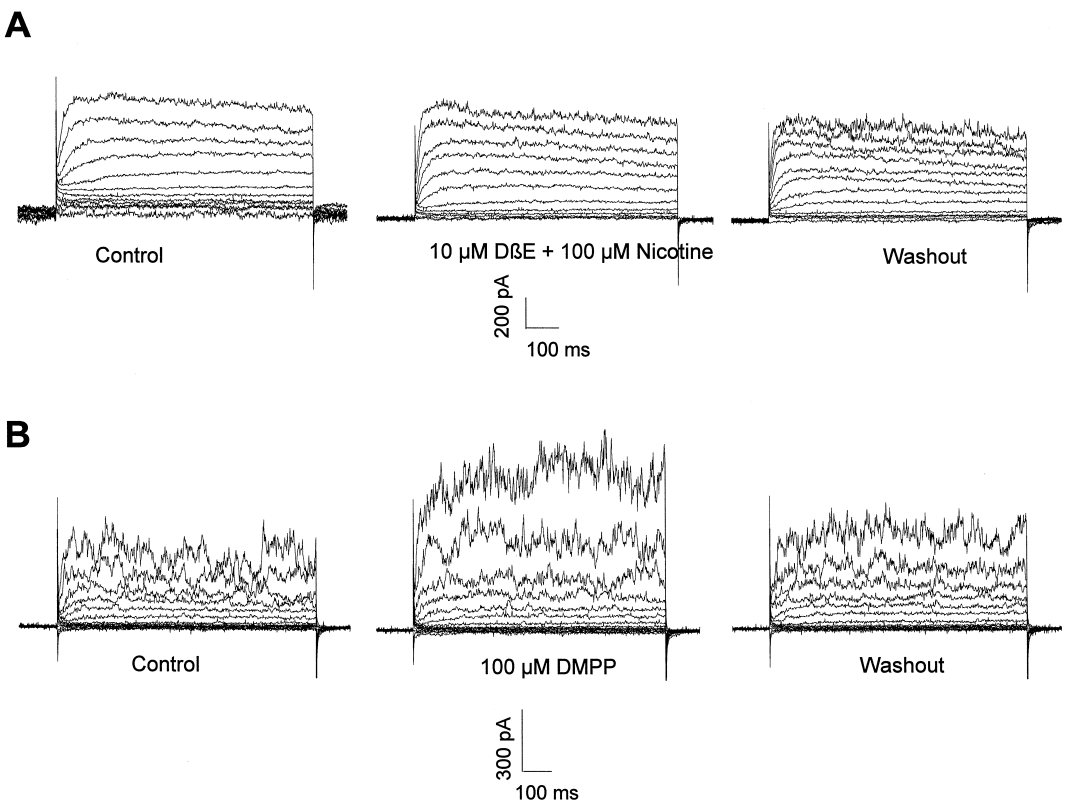

Fig. 7. The nicotinic receptor-dependent excitatory effect of nicotine on K^+ channel currents in rat tail artery smooth muscle cells. (A) The excitatory effect of nicotine (100 μM) on K^+ channel currents was abolished by pretreatment of the cell with 10 μM dihydro-β-erythroidine (DβE). (B) 1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP, 100 μM) directly increased K^+ channel currents in a reversible manner.

outcome of the blockade of K^+ channels. Unfortunately, these possibilities were not directly tested using the patch–clamp technique. Voltage-dependent Ca^{2+} channels (Koley et al., 1987; Damaj et al., 1993; Satoh, 1997) and Na^+ channels (Satoh and Nakatani, 1995; Marks et al., 1995) in ventricular myocytes or neurons are also subjected to nicotinic modulation. However, the effect of nicotine on ion channels in vascular smooth muscles has not been reported to date.

Our present study demonstrated that nicotine differentially affected K^+ channel currents in vascular smooth muscle cells. At low concentrations (1–100 μM), nicotine increased K^+ channel currents probably via the stimulation of nicotinic receptors. Additional evidence supporting this action mode is that the nicotinic receptor agonist, 1,1-dimethyl-4-phenylpiperazinium iodide, produced similar excitatory effect on K^+ channel currents. At high concentrations (0.3–3 mM), nicotine inhibited K^+ channel currents likely due to a direct effect on K^+ channel proteins for the inhibitory effect of nicotine cannot be abolished by nicotinic receptor antagonist. The dual effects of nicotine on K^+ channels in vascular smooth muscle cells echo a previous study on rat right atrium strips (Carryl et al., 1992). In that study, nicotine at concentrations lower than 60 μM had no effect on membrane potential. At 3 mM, nicotine initially shortened the action potential duration and depressed the overshoot within 15 s of the application. However, the action potential was prolonged 3 min after the application of nicotine. Nifedipine blocked the initial effect of nicotine on the overshoot of action potential while tetraethylammonium blocked the nicotine-induced prolongation of action potential. These observations led to the speculation that nicotine might initially directly block L-type Ca^{2+} channels and finally indirectly inhibit delayed rectifier K^+ channel currents in rat atrial cardiomyocytes.

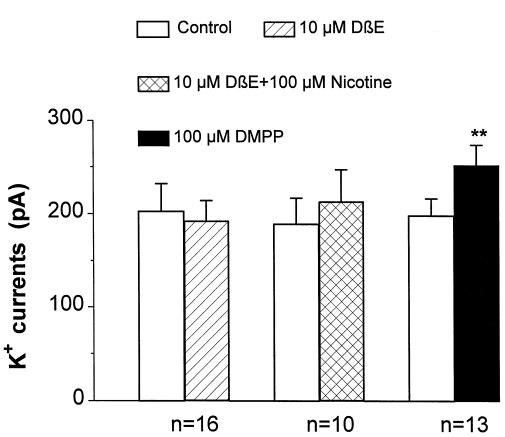

Fig. 8. Summary of the nicotinic receptor-dependent excitatory effect of nicotine on K^+ channel currents in rat tail artery smooth muscle cells. Test potential, +30 mV. $** P < 0.01$ vs. control.

In contrast, nicotine (100–300 μM) was reported to have no effect on the delayed rectifier K^+ channel current in rabbit sinoatrial nodal cells (Satoh, 1997). Variations in species, tissue types, and experimental conditions likely explain different effects of nicotine on K^+ channel currents observed in different experiments. Similarly, different cardiac effects of nicotine have been observed under different conditions, including positive (cat ventricular muscle, Bassett and Gelband, 1974), negative (rat heart, Carryl et al., 1992; Nakatani et al., 1994), or biphasic (guinea-pig atria, Pappano, 1970) chronotropic effects.

The nicotinic receptor-mediated excitatory effect on K^+ channel currents in our study may be secondary to the membrane depolarization induced by the stimulation of nicotinic receptors. In fact, our preliminary results showed that nicotine depolarized rat tail artery smooth muscle cells (unpublished observation). It is admitted that the presence of nicotinic receptors in vascular smooth muscle cells has not been confirmed. Our study took an indirect approach to show that nicotine might stimulate the smooth muscle nicotinic receptors, which may be different in their subunit composition from the nicotinic receptors in nerve systems, to activate K^+ channels. Regardless, the presence of neutral nicotinic receptors in cardiac muscle as well as skeletal muscle have been reported (Talib et al., 1993). The specific binding sites for nicotine in cerebral microvessels have been shown in human and pig (Kalaria et al., 1994), and the association of nicotinic receptors with cerebral microvessels was thus suggested. On the other hand, we also observed a nicotinic receptor-independent inhibitory effect of nicotine on K^+ channel currents. This mode of action might be as important as the nicotinic receptor-dependent effect according to our understanding of the cardiovascular responses to nicotine. One insight which can be perceived from this observation is that the absence of nicotinic receptors does not necessarily exclude the modulatory effect of nicotine on cellular or organ functions. The mechanisms underlying the dual effects of nicotine on K^+ channels in vascular smooth muscle cells may constitute one of the mechanisms responsible for the nicotine-induced vasorelaxation or vasoconstriction under different conditions.

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