Chronic Nicotine Alters NO Signaling of Ca$^{2+}$ Channels in Cerebral Arterioles

Volodymyr Gerzanich, Fangyi Zhang, G. Alexander West, J. Marc Simard

Abstract—Smoking is a major health hazard with proven deleterious effects on the cerebral circulation, including a decrease in cerebral blood flow and a high risk for stroke. To elucidate cellular mechanisms for the vasoconstrictive and pathological effects of nicotine, we used a nystatin-perforated patch-clamp technique to study Ca$^{2+}$ channels and Ca$^{2+}$-activated K$^+$ (BK) channels in smooth muscle cells isolated from cerebral lenticulostriate arterioles of rats chronically exposed to nicotine (4.5 mg/kg per day of nicotine free base, 15 to 22 days via osmotic minipump). Two major effects were observed in cells from nicotine-treated animals compared with controls. First, Ca$^{2+}$ channels were upregulated (0.48±0.03 pS/pF [20 cells] versus 0.35±0.01 pS/pF [31 cells], $P<0.005$) and BK channels were downregulated (12±3 pA/pF [14 cells] versus 34±7 pA/pF [14 cells], $P<0.05$), mimicking the effect of an apparent decrease in bioavailability of endogenous NO. Second, normal downregulation of Ca$^{2+}$ channels by exogenous NO (sodium nitroprusside [SNP], 100 nmol/L) and cGMP (8-bromo-cGMP, 0.1 mmol/L) was absent, whereas normal upregulation of BK channels by these agents was preserved, suggesting block of NO signaling downstream of cGMP-dependent protein kinase. In pial window preparations, chronic nicotine blunted NO-induced vasodilation of pial vessels and the increase in cortical blood flow measured by laser-Doppler flowmetry, demonstrating the importance of Ca$^{2+}$ channel downregulation in NO-induced vasorelaxation. These findings elucidate a new pathophysiological mechanism involving altered Ca$^{2+}$ homeostasis in cerebral arterioles that may predispose to stroke. (Circ Res. 2001;88: 359-365.)

Key Words: Ca$^{2+}$ channel • vascular smooth muscle • nicotine • nitric oxide • cerebral arteriole

Tobacco smoke, a health hazard most widely associated with cancer, is also a significant risk factor for stroke. Chronic exposure to tobacco smoke or to nicotine, a major vasoactive substance in tobacco, causes cerebral vasoconstriction and decreases cortical blood flow (CBF). Outside of the CNS, nicotine is well known to enhance vasoconstriction by impairing endothelium-dependent and endothelium-independent vasodilation. Vasoconstrictive effects of tobacco smoke and nicotine have been attributed to inhibition of Ca$^{2+}$ channels and K$^+$ channels by a mechanism heretofore undescribed, causing a block of normal downregulation of Ca$^{2+}$ channels by NO and cGMP without altering normal upregulation of Ca$^{2+}$-activated BK channels, via both voltage-dependent and voltage-independent mechanisms. Voltage-dependent inhibition arises from cGMP-dependent protein kinase (PKG) causing an increase in open probability of Ca$^{2+}$-activated K$^+$ (BK) channels, which polarize the cell and deactivate Ca$^{2+}$ channels. Voltage-independent inhibition arises from PKG phosphorylating the Ca$^{2+}$ channel itself or, more likely, a closely related regulatory phosphoprotein, thereby decreasing its open probability independently of voltage.

Given the important regulatory role of NO on ion channels, we postulated that impaired NO signaling expected with nicotine would increase availability of Ca$^{2+}$ channels and decrease availability of BK channels in smooth muscle cells. To test this hypothesis, we examined effects of chronic nicotine on availability of functional Ca$^{2+}$ channels and K$^+$ channels in lenticulostriate arteriolar smooth muscle cells (LSA-SMCs) of rats, vessels that in humans are preferentially involved in stroke. Also, to validate findings made on isolated cells, we examined the effects of chronic nicotine on vasomotor tone and on responses to NO in pial vessels in intact animals. Here we report that chronic exposure to nicotine, at concentrations comparable with those in humans who actively smoke cigarettes, caused 2 distinct effects who actively smoke cigarettes, 15 caused 2 distinct effects on availability of functional Ca$^{2+}$ channels and K$^+$ channels and decreased availability of BK channels in smooth muscle cells. To test this hypothesis, we examined effects of chronic nicotine on availability of functional Ca$^{2+}$ channels and K$^+$ channels in lenticulostriate arteriolar smooth muscle cells (LSA-SMCs) of rats, vessels that in humans are preferentially involved in stroke. Also, to validate findings made on isolated cells, we examined the effects of chronic nicotine on vasomotor tone and on responses to NO in pial vessels in intact animals. Here we report that chronic exposure to nicotine, at concentrations comparable with those in humans who actively smoke cigarettes, caused 2 distinct effects related to smooth muscle ion channel regulation. First, nicotine increased availability of Ca$^{2+}$ channels and decreased availability of Ca$^{2+}$-activated K$^+$ channels in cerebral arterioles. Secondly, it altered NO signaling of L-type Ca$^{2+}$ channels by a mechanism heretofore undescribed, causing a block of normal downregulation of Ca$^{2+}$ channels by NO and cGMP without altering normal upregulation of Ca$^{2+}$-activated BK channels, via both voltage-dependent and voltage-independent mechanisms. Tobacco smoke, a health hazard most widely associated with cancer, is also a significant risk factor for stroke. Chronic exposure to tobacco smoke or to nicotine, a major vasoactive substance in tobacco, causes cerebral vasoconstriction and decreases cortical blood flow (CBF). Outside of the CNS, nicotine is well known to enhance vasoconstriction by impairing endothelium-dependent and endothelium-independent vasodilation. Vasoconstrictive effects of tobacco smoke and nicotine have been attributed to inhibition of Ca$^{2+}$ channels and K$^+$ channels by a mechanism heretofore undescribed, causing a block of normal downregulation of Ca$^{2+}$ channels by NO and cGMP without altering normal upregulation of Ca$^{2+}$-activated BK channels, via both voltage-dependent and voltage-independent mechanisms. Tobacco smoke, a health hazard most widely associated with cancer, is also a significant risk factor for stroke. Chronic exposure to tobacco smoke or to nicotine, a major vasoactive substance in tobacco, causes cerebral vasoconstriction and decreases cortical blood flow (CBF). Outside of the CNS, nicotine is well known to enhance vasoconstriction by impairing endothelium-dependent and endothelium-independent vasodilation. Vasoconstrictive effects of tobacco smoke and nicotine have been attributed to inhibition of Ca$^{2+}$ channels and K$^+$ channels by a mechanism heretofore undescribed, causing a block of normal downregulation of Ca$^{2+}$ channels by NO and cGMP without altering normal upregulation of Ca$^{2+}$-activated BK channels, via both voltage-dependent and voltage-independent mechanisms.
K\(^+\) channels by NO and cGMP. Moreover, the significance of these ion channel effects was corroborated by showing reduced pial vasorelaxation in response to NO in animals chronically exposed to nicotine. These novel effects of nicotine, not previously reported for any chemical agent, provide new insight into the signaling mechanism used by NO to achieve vasorelaxation and provide a mechanism for altered Ca\(^{2+}\) homeostasis leading to pathological effects that may predispose to stroke.

**Materials and Methods**

**Animal Model**

Female Wistar-Kyoto rats, 220 to 300 g, were implanted with osmotic minipumps (Alzet 2002, Alza Corp) that delivered 0.9% NaCl without or with nicotine tartrate (Sigma; 4.5 mg/kg per day of nicotine free base) for 15 to 22 days, yielding plasma levels of nicotine (500 nmol/L)\(^{16,17}\) comparable with those in smokers. \(^{15}\) Animals exposed to nicotine showed normal growth, weight, and blood pressure.

**Laser Doppler Flowmetry (LDF) and Pial Vessel Diameter**

Experiments with either saline pump- or nicotine pump-implanted rats were blinded to the experimenters. An animal was anesthetized with 1% halothane. The right femoral artery and vein were cannulated for monitoring blood pressure, blood gas sampling, and intravenous drug administration. The animal was tracheostomized, immobilized with tubocurarine chloride (1 mg/kg IV), and mechanically ventilated to maintain physiological blood gas tensions. Rectal temperature was maintained at 37°C. The animal was secured in a stereotactic frame, and the skull was exposed through a longitudinal incision for preparation of a closed cranial window. \(^{18}\) The cranial window was continuously superfused at 0.5 mL/min with artificial cerebrospinal fluid equilibrated to maintain normal cerebrospinal fluid pH and gas tension as well as intracranial pressure. Pial vessel diameter was continuously monitored using a videomicroscopy system (Microcirculation Research Institute, Texas A&M University Health Science Center). CBF was measured simultaneously in the contralateral hemisphere using LDF (TSI Inc) via an open cranial window. \(^{19}\) The laser-Doppler probe (0.8 mm) was placed contralateral hemisphere using LDF (TSI Inc) via an open cranial window. To quantify Ca\(^{2+}\) channel availability, current-voltage data from individual cells between −40 V and +40 mV were fit to the Boltzmann function:

\[
I = g_{\text{max}} \cdot (E - E_{rev}) / [1 + \exp((E_{0.5} - E)/k)],
\]

where \(I\) is current; \(E\) is membrane potential; \(g_{\text{max}}\) is the maximum conductance at positive potentials; \(k\) and \(E_{0.5}\) are the steepness and midpoint potential of activation, respectively; and \(E_{rev}\) is the extrapolated reversal potential for the chord conductance. Ca\(^{2+}\) channel availability was then calculated as normalized maximum conductance, \(g_{\text{max}}/g_{\text{cell}}\), obtained by dividing \(g_{\text{max}}\) by cell capacitance.

Data were fit to the equation using the iterative nonlinear, least-squares method of Marquardt-Levenberg (Origin 6.0, Microcal). Statistical comparisons were evaluated using Student \(t\) test. All data are given as mean±SE.

**Results**

We first characterized the Ca\(^{2+}\) channels in LSA-SMCs because they had not been previously described. When recorded with 10 mmol/L Ba\(^{2+}\) as the charge carrier, the macroscopic inward currents showed kinetic and voltage-dependent properties that are typical for L-type Ca\(^{2+}\) channels (Figures 1A and 1Ca). The activating dihydropyridine, Bay k8644, doubled the current (Figure 1Cb), and the blocking dihydropyridine, nifedipine, blocked most of the current (Figure 1Cc). Recordings of cell-attached patches studied with 40 mmol/L Ba\(^{2+}\) plus Bay k8644 in the pipette solution revealed single-channel inward currents (Figure 1E, a through c) with a slope conductance of 22 pS (Figure 1F, open circles) with a slope conductance of 22 pS (Figure 1F, open circles). Together, these findings are consistent with previous reports on Ca\(^{2+}\) channels in other vascular smooth muscle cells\(^{22,23}\) and confirm that LSA-SMCs express predominantly a single class of L-type, dihydropyridine-sensitive Ca\(^{2+}\) channels. As previously observed in cerebral smooth muscle, no evidence was found for T-type Ca\(^{2+}\) channels in these cells. \(^{24}\)

In cells from rats chronically exposed to nicotine, the biophysical and pharmacological properties of the Ca\(^{2+}\) channel currents were indistinguishable from those of controls. Macroscopic inward currents showed kinetic and voltage-dependent properties typical for L-type Ca\(^{2+}\) channels (Figures 1B and 1Da). The activating dihydropyridine, Bay k8644, increased the current (Figure 1Db), and the blocking dihydropyridine, nifedipine, blocked it (Figure 1Dc). Recordings of cell-attached patches revealed single-channel inward currents (Figure 1E, d–f) with a slope conductance of 22 pS (Figure 1F, open circles).
Figure 1. Chronic nicotine upregulates Ca\(^{2+}\) channels in LSA-SMCs. A and B, Ca\(^{2+}\) channel currents during step pulses from −40 to +40 mV in LSA-SMCs from a control animal (A, 12 pF) and from a chronic nicotine animal (B, 13 pF). C and D, Current-voltage curves recorded during ramp pulses in LSA-SMCs from a control animal (C, 9 pF) and from a chronic nicotine animal (D, 14 pF) before (a) and after 1 μmol/L Bay k8644 (b) and 1 μmol/L nifedipine (c). E, Single-channel currents, with inward cationic currents plotted downward, in cell-attached patches from a control animal (a through c) and from a chronic nicotine animal (d through f), recorded at E=−30 mV (a and d), −40 mV (b and e), and −50 mV (c and f), with Bay k8644 and 40 mmol/L Ba\(^{2+}\) in the pipette solution. F, Open-channel current vs membrane potential showing 22-pS conductance in 5 membrane patches from control (a) and 5 membrane patches from chronic nicotine (c) animals. G, Average (±SE) current-voltage curves recorded during ramp pulses in LSA-SMCs from control (a, 25 cells; capacitance, 11.8±0.6 pF) and from chronic nicotine (b, 14 cells; capacitance, 11.2±0.8 pF) animals. H, Normalized values for Ca\(^{2+}\) channel availability in LSA-SMCs were obtained by fitting to a Boltzmann equation to obtain g\(_{\text{max}}\) and dividing by cell capacitance. Values from control animals (CONT) and chronic nicotine (NIC) animals, 0.35±0.01 pS/pF (31 cells) and 0.48±0.03 pS/pF (20 cells) respectively, were significantly different by t test (P<0.05). Fit to Boltzmann equation gave voltage-dependent parameters of E\(_{1/2}=-3.6±0.8\) mV and −2.4±1.1 mV and k=5.6±0.2 and 5.1±0.2 mV for the control and nicotine groups, respectively, which were not significantly different by t test (P>0.05).

However, in cells from rats chronically exposed to nicotine, the magnitude of the Ca\(^{2+}\) channel current was appreciably larger than in cells from control animals (Figure 1G). Current-voltage curves for individual cells were fit to a Boltzmann function (Equation) to quantify channel availability, revealing that normalized values in cells from nicotine-treated rats were significantly elevated (Figure 1H), although they showed no change in voltage dependence (see legend, Figure 1). Thus, chronic exposure to nicotine significantly augmented availability of Ca\(^{2+}\) channels without causing any change in kinetic or voltage-dependent properties, single-channel conductance, or pharmacological response to dihydropyridines.

In smooth muscle, outward K\(^+\) currents regulate Ca\(^{2+}\) channels by polarizing the cell and deactivating Ca\(^{2+}\) channels. We recorded outward currents in LSA-SMCs at different voltages (Figure 2A). The outward current was dominated by BK channels, as indicated by its insensitivity to glibenclamide, minimal block by 4-aminopyridine, and high sensitivity to iberiotoxin and charybdotoxin (Figure 2C).\(^{25}\) In cells from chronic nicotine rats, electrophysiological and pharmacological properties, including kinetics, voltage dependence (Figure 2B), and sensitivity to iberiotoxin and charybdotoxin (Figure 2D), were similar to those of controls, which suggests no change in types of channels expressed. However, the magnitude of the current was significantly smaller in cells from chronic nicotine animals (Figure 2E). Thus, chronic exposure to nicotine resulted not only in upregulation of Ca\(^{2+}\) channels but also in downregulation of BK channels.

NO is known to downregulate Ca\(^{2+}\) channels and upregulate BK channels.\(^{9-12}\) We thus interpreted our findings of augmented Ca\(^{2+}\) channel availability and decreased BK channel availability with nicotine as being consistent with an apparent decrease in bioavailability of endogenous NO. This hypothesis accorded with our recent finding that block of endogenous NOS activity, as well as acute endothelial injury, results in upregulation of Ca\(^{2+}\) channels in smooth muscle cells.\(^{21}\) Because we also found in the same report that block of endogenous NO augments the apparent efficacy of exogenous NO in downregulating Ca\(^{2+}\) channels, we sought here to determine whether chronic nicotine also would cause an increase in apparent efficacy of NO.

Cells were studied using 100 nmol/L of the NO donor sodium nitroprusside (SNP), a concentration that causes maximum downregulation of Ca\(^{2+}\) channel currents.\(^{21}\) The effects of SNP in LSA-SMCs from control animals were comparable with previous observations in vascular smooth muscle, with a gradual decrease in whole-cell current (Figure 2). NO treatment did not alter the inward Ca\(^{2+}\) channel currents (Figure 2E), providing additional support for the hypothesis that chronic nicotine downregulated Ca\(^{2+}\) channels enogenous NO in downregulating Ca\(^{2+}\) channels.
Figure 3. Chronic nicotine blocks downregulation of Ca\(^{2+}\) channels by NO and cGMP. A and D, Current-voltage curves recorded during ramp pulse in LSA-SMCs from control animals before (a) and after (b) 100 nmol/L SNP (A) and before (a) and after (b) 100 μmol/L 8-bromo-cGMP (D). B and E, Current-voltage curves recorded during ramp pulse in LSA-SMCs from chronic nicotine animals before (a) and after (b) 100 nmol/L SNP (B) and before (a) and after (b) 100 μmol/L 8-bromo-cGMP (E). C and F, Effect of 100 nmol/L SNP (C) and 100 μmol/L 8-bromo-cGMP (8Br-cGMP) (F) on peak Ca\(^{2+}\) channel currents in LSA-SMCs from control (●) and chronic nicotine (○) animals; values (mean±SE) are plotted for 9 and 8 cells for SNP and for 6 and 7 cells for 8-bromo-cGMP, for control and chronic nicotine animals, respectively. 

3C, closed circles) and no effect on voltage dependence (Figure 3A). The effect of NO donor on Ca\(^{2+}\) channel currents in cells from chronic nicotine rats was unexpected. In contrast to downregulation observed in cells from control animals (Figures 3A and 3C, closed circles), from other smooth muscle preparations, and from vessels with simple endothelial injury, application of 100 nmol/L SNP to cells from rats exposed to nicotine caused no change in the current during a 10-minute or longer exposure (Figures 3B and 3C, open circles). Thus, chronic exposure to nicotine abolished the inhibitory effect of exogenous NO on Ca\(^{2+}\) channel currents.

In smooth muscle, effects of NO are mediated by cGMP. With cells from control animals, application of the membrane-permeable analogue, 8-bromo-cGMP, caused the expected downregulation of Ca\(^{2+}\) channel current (Figures 3D and 3F, closed circles). By contrast, in cells from chronic nicotine rats, 8-bromo-cGMP had no effect on Ca\(^{2+}\) channel currents (Figure 3E and 3F, open circles), corroborating the result obtained with NO donor.

NO and cGMP also upregulate BK channels. In cells from control rats, SNP (Figure 4A) and 8-bromo-cGMP caused a robust increase in outward current. In cells from chronic nicotine rats, both SNP (Figure 4B) and 8-bromo-cGMP (Figure 4C) increased the outward current in a manner indistinguishable from controls. Together, these findings with BK channels suggest that neither guanylate cyclase nor PKG was affected by nicotine, suggesting that the location for the block of NO- and cGMP-mediated downregulation of Ca\(^{2+}\) channels by nicotine was downstream of PKG.

The data presented are consistent with the hypothesis that nicotine exerted complex effects by at least 2 mechanisms, as follows: (1) by apparent reduction in bioavailability of endogenous NO and (2) by interfering with NO signaling in smooth muscle. Exposure to nicotine also causes release of endothelin, although not in cerebral vascular smooth muscle, endothelin can upregulate Ca\(^{2+}\) channels. In LSA-SMCs, endothelin (0.1 to 100 nmol/L) caused no increase in current (11 cells). Also, to exclude possible direct stimulatory effects on Ca\(^{2+}\) channels or inhibitory effects on BK channels, we applied nicotine directly to cells from control animals. Application of nicotine (0.5 and 10 μmol/L) for 5 to 12 minutes caused no change in Ca\(^{2+}\) channels (9 cells) or BK (5 cells) channel current. Also, preexposure of cells to nicotine in vitro did not prevent subsequent normal downregulation of Ca\(^{2+}\) channel current (8 cells), indicating that nicotine was not acting as an NO scavenger.

Finally, application of the tyrosine kinase inhibitor, tyrophostin (AG-18), resulted in significant and equivalent downregulation of Ca\(^{2+}\) channels in LSA-SMCs from both control (Figure 4D) and chronic nicotine (Figure 4E) rats, suggesting that loss of the response to NO/cGMP was not due to a nonspecific effect preventing phosphorylation or dephosphorylation of the channel. Together, these control experiments showed that altered channel availability observed with chronic nicotine was not due to either endothelin release or to direct effects of nicotine on Ca\(^{2+}\) or BK channels and that loss of NO signaling was not due to nonspecific effects on Ca\(^{2+}\) channels.

Finally, we sought to determine whether findings in isolated cells would predict effects in intact cerebral arterioles. We used LDF to measure changes in CBF and a pial window technique to measure pial arteriolar diameter. In control rats, infusion of the NO donor S-nitroso-N-acetylpenicillamine (SNAP) caused a progressive increase in CBF (Figures 5Aa and 5B CONT) and in pial vessel diameter (Figure 5C CONT). By contrast, in chronic nicotine rats, the same protocol with SNAP resulted in significantly less vasodilation, as measured by both CBF and pial vessel diameter (Figures 5Ab, 5B NIC, and 5C NIC). The finding of a significant but incomplete block of NO effect in arterioles in vivo expands on previous observations of effects of nicotine.
in noncerebral vessels and corroborates the observation in isolated cells that chronic nicotine blocks NO effects involving Ca²⁺ but not BK channels, suggesting that both channels must be involved for full relaxation.

Discussion

Chronic exposure to nicotine at concentrations comparable with those in humans who actively smoke resulted in 2 major types of effects on smooth muscle ion channels, as follows: (1) an alteration in ion channel availability, with Ca²⁺ channels upregulated and BK channels downregulated, and (2) a block in NO-mediated downregulation of Ca²⁺ channels but not of NO-mediated upregulation of BK channels.

An increase in availability of Ca²⁺ channels can arise by a variety of mechanisms, including a change in biophysical properties of the channel, a second messenger–mediated increase in open probability or open time of the channel, or an increase in expression of functional channels. The first of these was excluded by our data showing no effect of chronic nicotine on the biophysical or pharmacological properties of the Ca²⁺ channel. Although we have no data specifically excluding an increase in expression of Ca²⁺ channels, at present we favor an alteration in second messenger–mediated regulation as the most parsimonious explanation for both simultaneous upregulation of Ca²⁺ channels and downregulation of BK channels. Because NO normally downregulates Ca²⁺ channels and upregulates BK channels, the observed upregulation of Ca²⁺ channels and downregulation of BK channels mimics precisely the effects expected with an apparent decrease in bioavailability of endogenous NO. Indeed, we recently demonstrated that a decrease in bioavailability of endogenous NO, as well as block of endogenous NOS activity and oxidative endothelial injury, results in a significant increase in Ca²⁺ channel availability, similar to that observed here with chronic nicotine. Nicotine is known to cause desquamation and ultrastructural abnormalities in endothelium and vasoconstrictive effects of nicotine have been postulated to be due to impaired release of NO, possibly as a result of oxidative stress. Thus, endothelial dysfunction, possibly initiated via endothelial nicotinic receptors, would account well for the altered channel availability observed here. Additional work will be required to confirm by direct measurement the apparent reduction in bioavailability of endogenous NO in the smooth muscle layer of arterioles in vivo that is suggested by our data.

The second major effect of nicotine was the novel finding of a block of normal NO-mediated downregulation of Ca²⁺ channels. Our experiments not only established that nicotine blocks NO signaling involving Ca²⁺ channels, but they also helped localize the site of block in the signaling pathway. A critical experiment was the one showing maintenance of the effect of NO on BK channel upregulation. This finding essentially eliminates the initial steps in the signaling pathway, including guanylate cyclase, cGMP, or PKG, and points to a site downstream of PKG as the target of nicotine. An effect downstream of PKG suggests either that the channel itself is altered by nicotine, resulting in diminished sensitivity to PKG phosphorylation, or that the channel is not phosphorylated by PKG. Our experiments showing no effect of chronic nicotine on the biophysical or pharmacological properties of the Ca²⁺ channel provide no support for a hypothesis of an altered channel, and similarly our experiment showing no effect of chronic nicotine on downregulation with tyrosine kinase inhibitor argues that phosphorylation mechanisms directly involving the channel are not affected by nicotine. Alternatively, if the channel itself is not altered by nicotine, this would suggest that an intermediate regulatory phosphoprotein may be interposed between PKG and the channel and that this intermediate phosphoprotein is the target of nicotine. At present, the specific phosphoprotein target of PKG involved in NO-mediated downregulation of Ca²⁺ channels in smooth muscle is not known. In cardiac cells, Ca²⁺ channels are phosphorylated by PKG, resulting in a reduced open probability. L-type Ca²⁺ channels are heteropentameric complexes, with the α₁ subunit subject to phosphorylation by PKG. The molecular diversity of α₁ genes and the splice variants produced from these genes is extensive, however, raising the possibility that the α₁ subunit in cerebrovascular smooth muscle, unlike cardiac cells, may not be phosphorylated by PKG, or if it is, that phosphorylation does not cause a decrease in open probability. Further work will be required to clarify whether nicotine directly alters the Ca²⁺ channel to block PKG phosphorylation or whether nicotine blocks a putative intermediate regulatory phosphoprotein downstream of PKG that is involved in downregulating the channel.

Inhibition of Ca²⁺ influx is critical to relaxation of cerebrovascular smooth muscle. Thus, 2 distinct signaling mechanisms initiated by NO have evolved to inhibit Ca²⁺ influx. One of these is the signaling pathway that involves PKG-mediated phosphorylation of BK channels (Figure 6, lower branch). This pathway is voltage dependent, because the regulatory phosphoprotein, the BK channel, is coupled to its target, the Ca²⁺ channel, by virtue of the intrinsic voltage dependence of the Ca²⁺ channel. PKG-mediated phosphorylation of the BK channel increases its availability, serving to
Figure 6. NO inhibits L-type Ca$^{2+}$ channel by 2 parallel mechanisms. Lower branch, Voltage-dependent, iberiotoxin-sensitive mechanism using BK channel as an intermediate regulatory phosphoprotein acting on the Ca$^{2+}$ channel. Upper branch, Voltage-independent, nicotine-sensitive mechanism using unknown regulatory phosphoprotein (7) to act on the Ca$^{2+}$ channel. PKG indicates cGMP-dependent protein kinase; Ca CHAN, Ca$^{2+}$ channel; and $\bigcirc$, downregulation.

polarize the cell and thereby turn off voltage-dependent Ca$^{2+}$ channels and decrease Ca$^{2+}$ influx. This pathway has been extensively investigated,37,38 given the wide availability of $K^+$ channel blockers.

Less well understood is a second signaling pathway involving PKG-mediated downregulation of Ca$^{2+}$ channels11,12 that is independent of $K^+$ channels (Figure 6, upper branch). As noted above, the specific target of PKG in this branch remains to be identified, but our data suggest that it is this step involving action of an unidentified regulatory phosphoprotein on the Ca$^{2+}$ channel that may be blocked either directly or indirectly by nicotine (Figure 6, upper branch). This pathway is voltage independent, given that the intrinsic voltage dependence of the Ca$^{2+}$ channel is not involved in coupling the regulatory phosphoprotein to its target, the Ca$^{2+}$ channel. The unique effect of nicotine reported here aids in delineating the specific contribution of the voltage-independent mechanism to the process of vasorelaxation, showing that approximately half of the vasodilatory response to NO in vivo was eliminated when the voltage-independent mechanism was blocked by nicotine. The present study using nicotine is the first to assess the important contribution of L-type Ca$^{2+}$ channel inhibition, independent of BK channel–mediated voltage-dependent deactivation, in achieving NO-induced vasorelaxation.

The effects of chronic nicotine reported here provide both a mechanistic basis for abnormal vasorelaxation and a likely explanation for structural pathological effects. Increased availability of Ca$^{2+}$ channels favors increased basal Ca$^{2+}$ influx into smooth muscle cells that, if uncompensated, will lead to Ca$^{2+}$-induced cell injury and cell death.39 Tobacco smoke leads to numerous degenerative changes in cerebral vessels, including intimal hyperplasia, atherosclerosis, loss of smooth muscle cells, and aneurysm formation.40 These pathological changes have been attributed to activation of matrix metalloproteinases, increased DNA synthesis, and cell proliferation.41 The data presented here indicate that nicotine-induced alteration of Ca$^{2+}$ homeostasis may be responsible for smooth muscle cell toxicity and cell death.

Acknowledgments

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References


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