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Altered behavioral sensitivity of Ca^{2+}-modulating drugs after chronic nicotine administration in mice

M. Imad Damaj *

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613, USA

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Abstract

Numerous studies have demonstrated that tolerance develops to the physiological and behavioral effects of nicotine in animals after chronic administration of the drug. However, the mechanisms underlying tolerance to nicotine are not well known. There are several lines of evidence which support a role for Ca^{2+} in nicotine's acute pharmacological effects. The objective of the study was to determine whether Ca^{2+} plays a role in the development of tolerance to nicotine by investigating the behavioral activity of several Ca^{2+} modulating drugs after systemic (BAY K 8644: (±)-1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester) and intrathecal administration (BAY K 8644, Ca^{2+} and thapsigargin) in nicotine-tolerant mice. The ability of BAY K 8644 to induce motor impairment and hypomotility after i.p. injection was decreased in nicotine-tolerant mice. In addition, tolerance to Ca^{2+}, thapsigargin, and BAY K 8644-induced antinociception after i.t. injection also developed in nicotine-tolerant mice. ED_{50} values for BAY K 8644 and thapsigargin increased from 3.7 to 12 μg/mouse and 0.83 to 19.7 μg/mouse, respectively. The greatest tolerance developed to the effects of thapsigargin with an ED_{50} value that increased from 0.83 to 20 μg. Furthermore, chronic nicotine injections did not alter [^{3}H]nitrendipine binding in the brain. These results suggest the involvement of Ca^{2+}-dependent mechanisms in nicotine tolerance in mice.

Keywords: Nicotine; Antinociception; Ca^{2+}; Ca^{2+} channel; Tolerance; Thapsigargin; (Inthrathecal)

1. Introduction

Tobacco use, especially when smoked as cigarettes, remains a major avoidable cause of mortality and morbidity in the western world. Although many factors contribute to the overall effects of smoking, there is convincing evidence that smoking behavior is maintained by the pharmacological effects of the tobacco alkaloid nicotine (Surgeon, 1988). Humans become physically dependent upon and tolerant to nicotine, and it has been suggested that these processes contribute to the difficulty smokers have in stopping cigarette use (Benowitz, 1992). Numerous studies have demonstrated that tolerance develops to the physiological and behavioral effects of nicotine in animals after chronic administration of the drug. Following chronic treatment with nicotine, animals exhibit decreases in sensitivity to acute challenges with the drug in numerous physiological and behavioral measures, including respiration, locomotor activity, body temperature, sensitivity to seizures (Marks et al., 1993, 1986) and antinociception (Damaj and Martin, 1996). The mechanisms underlying tolerance to nicotine are not well known. Development of tolerance was thought to be related to changes in nicotinic receptor binding after chronic exposure of the drug. Indeed, repeated exposure to nicotine produces significant increases in the number of [^{3}H]nicotine binding sites in several rat and mouse brain regions (Marks et al., 1986; Schwartz and Kellar, 1985). Receptor desensitization has been suggested to be the primary event leading to up-regulation of central nervous system (CNS) nicotinic receptors and a compensatory response to chronic desensitization following prolonged agonist treatment. In M10 cells, it has been shown that a decrease in receptor turnover accounts for the increased number of $\alpha_4\beta_2$ receptors (Peng et al., 1994). However, little relationship has been found between the binding affinity of several nicotinic receptor ligands (nicotine, lobeline, anabasine) and their ability to induce receptor up-regulation (Bhat et al., 1991). In addition, several reports suggest that tolerance to nicotine cannot be completely explained by changes in receptor number

* Tel.: (1-804) 828-1676; Fax: (1-804) 828-2117.

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(Marks et al., 1987; Pauly et al., 1992). It is well known that tolerance to psychoactive drugs like nicotine may involve neuronal adaptation not only at the level of the drug-receptor interaction, but post-receptor events such as receptor coupling and intracellular second and third messenger cascades.

Of particular interest are Ca^{2+} ions that play a major role in signal transduction events. There are several lines of evidence which support a role for Ca^{2+} in the signaling process of the nicotine receptor. Nicotine-induced release of neurotransmitters in the brain is largely Ca^{2+}-dependent (Rapier et al., 1988; Rowell and Winkler, 1984) and some of the neuronal nicotinic receptor subtypes are Ca^{2+}-permeable (Barrantes et al., 1995; Castro and Albuquerque, 1995; Vernino et al., 1992). Recently, we showed that voltage-dependent Ca^{2+} channel blockers and activators were able to modulate nicotine's acute effects on locomotor activity and antinociception (tail-flick test) in mice (Damaj et al., 1993; Damaj and Martin, 1993).

The objective of this study was to determine whether tolerance to nicotine's pharmacological effects involves Ca^{2+}-dependent mechanisms by investigating the behavioral activity of several Ca^{2+}-modulating drugs after systemic (BAY K 8644) and intrathecal administration (BAY K 8644, Ca^{2+} and thapsigargin) in nicotine-tolerant mice. Nicotine-tolerant mice were used to assess the pharmacological effects of drugs which modulate Ca^{2+} fluxes by acting on different targets: extracellular Ca^{2+} (voltage-gated ion channels), thapsigargin (intracellular Ca^{2+} stores) and BAY K 8644 (L-type Ca^{2+} channels) and to determine whether changes occurred in [^{3}H]nitrendipine binding.

2. Materials and methods

2.1. Animals

Male ICR mice (20–25 g) obtained from Harlan Laboratories (Indianapolis, IN, USA) were used throughout the study. They were housed in groups of six and had free access to food and water. Animals were housed in an American Association for Accreditation of Laboratory Animal Care approved facility and the study was approved by the Institutional Animal Care and Use Committee of Virginia Commomwealth University.

2.2. Drugs

(−)-Nicotine was obtained from Aldrich (Milwaukee, WI, USA) and converted to the ditartrate salt as described by Aceto et al. (1979). Other drugs were obtained as follows: Calcium chloride was obtained from Sigma (St. Louis, MO, USA). (±)-1,4-Dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester (BAY K 8644) was obtained from Research Biochemical International (Natick, MA, USA), and thapsigargin was purchased from LC Services (Woburn, MA, USA). [^{3}H]Nitrendipine (specific activity, 82.3 Ci/mmol) was obtained from New England Nuclear-Dupont (Boston, MA, USA). Nicotine ditartrate and calcium chloride were dissolved in physiological saline (0.9% sodium chloride). Thapsigargin and BAY K 8644 were prepared in dimethylsulfoxide (DMSO). Solutions of BAY K 8644 were refrigerated in foil-lined containers. For locomotor activity and body temperature experiment, BAY K 8644 was prepared in emulphor/ethanol/saline (1:1:18). Emulphor (EL620) was obtained from Rhone Poulenc (Crambury, NJ, USA). Drugs were given in a total volume of 1 ml/100 g body weight in mice. All doses were expressed as the free base of the drug.

2.3. Intrathecal injections

Intrathecal (i.t.) injections were performed free-hand between the L5 and L6 lumbar space in unanesthetized male mice according to the method of Hylden and Wilcox (1980). The injection was performed using a 30-gauge needle attached to a glass microsyringe. The injection volume in all cases was 5 μl. The accurate placement of the needle was evidenced by a quick 'flick' of the mouse's tail.

2.4. Pharmacological assays in mice

2.4.1. Antinociception

Antinociception in mice was measured by the tail-flick method of D'Amour and Smith (1941) as modified by Dewey et al. (1970). Groups of six animals were used for each dose and for each treatment. A control response (2–4 s) was determined for each animal before treatment, and test latencies were assessed at various times after drug administration. A maximum latency of 10 s was imposed if no response occurred within that time. Antinociceptive response was calculated as %MPE (maximum possible effect), where $\%MPE = [(test - control)/(10 - control) \times 100]$. Nicotine-tolerant mice were challenged acutely with an i.t. injection of either nicotine, Ca^{2+}, thapsigargin or BAY K 8644. The animals were tested either 5 min after thapsigargin, nicotine and BAY K 8644, or 20 min after Ca^{2+}.

2.4.2. Motor coordination

In order to measure the effects of BAY K 8644 on motor coordination, a wooden rod 6 cm in diameter was partitioned into three compartments by circular metal discs (28 cm in diameter) at 18-cm intervals. The rod was attached to a motor and rotated at a rate of 4 rpm. Naive mice were trained until they could remain on the rotarod for 3 min. Animals that failed to meet this criterion within five trials were discarded. This training took place no longer than 15 min before the subcutaneous (s.c.) adminis-

tration of nicotine. Thirty minutes after i.p. injection of BAY K 8644, mice were placed on the rotarod for 5 min. The amount of time the animals remained on the rotarod was recorded, and percent impairment was calculated as % impairment = [(1 − (test time in seconds/300)) × 100]. Impairment of 0% corresponds to the subjects that remained on the rotarod for 5 min (300 s) and 100% impairment corresponds to subjects that fell off the rotarod in less than 1 s.

2.4.3. Locomotor activity

Mice were placed into individual Omnitech photocell activity cages (28 × 16.5 cm) 5 min after i.p. administration of either vehicle or BAY K 8644. Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 10 min. Data were expressed as the number of photocell interruptions.

Eight to twelve mice were tested in each treatment group and each animal was tested only once.

2.5. Chronic drug treatment paradigms

Two groups of animals received s.c. injections of either (−)- nicotine (2 mg/kg) or saline twice daily (08:30 and 16:30 h) for 10 days. During the treatment period the body weight was recorded every other day. At day 11, mice were challenged with i.t. injections of nicotine, Ca^{2+}, thapsigargin or BAY K 8644 for determination of dose-response curves in the tail-flick test. Injections and testing procedures were performed in the same room. Another group of mice was challenged i.p. with BAY K 8644, and the effects on locomotor activity and motor coordination were measured.

2.6. Binding of [^{3}H]nitrendipine to mouse brain

Saline or nicotine chronically treated mice were decapitated 12 h after the last injection, and their brains were removed and homogenized in 10 vols., respectively, of 50 mM Tris-HCl (pH 7.4). Samples were centrifuged at 3000 × *g* for 10 min, and the supernatant collected and centrifuged at 16 500 × *g* for 30 min. The pellet was resuspended in Tris-HCl to achieve protein concentrations of 1.75–2.25 mg/ml. Membranes (200 μl) were incubated in 50 mM Tris-HCl containing 0.1% bovine serum albumin (BSA) and a final concentration of 3.75% ethanol, with [^{3}H]nitrendipine ranging from 2 nM to 32 nM for 90 min at 22°C. Filters were soaked in 0.1% PEI and placed in a Brandel Cell Harvester M-48R (Gaithersburg, MD, USA) before assay buffer was rapidly filtered under vacuum. The tubes and harvester were washed twice with ice-cold Tris-HCl buffer, and the filter circles removed and added to cocktail in scintillation vials for determination of radioactivity. Nifedipine (1 μM) was used to determine nonspecific binding. Data were analyzed using the EBDA program for Macintosh to determine K_d and B_{max}.

2.7. Statistical analysis

ED_{50} values with 95% confidence limits (CL) for antinociception and motor impairment data were calculated by unweighted least-squares linear regression for log-doses vs. probits, as described by Tallarida and Murray (1987).

3. Results

3.1. Effects of BAY K 8644 on locomotor activity and motor coordination in nicotine-tolerant mice

Mice chronically treated with s.c. nicotine were challenged with different doses of nicotine and BAY K 8644 (i.p. administration) and evaluated for motor coordination and locomotor activity. Mice became tolerant to nictotine-induced hypomotility and motor impairment as shown by the rightward shift of the dose-response curve after treatment with chronic nicotine (Fig. 1A, B), and the ED_{50} values increased from 1.1 (0.6–2.1) and 0.50 (0.2–0.75) in saline-treated animals to 3.0 (2.2–4.3) and 2.1 (1.5–2.8) mg/kg in nicotine-tolerant mice for nicotine-induced motor impairment and hypomotility, respectively. Furthermore, the ability of BAY K 8644 to induce motor impairment and hypomotility was decreased in nicotine-treated

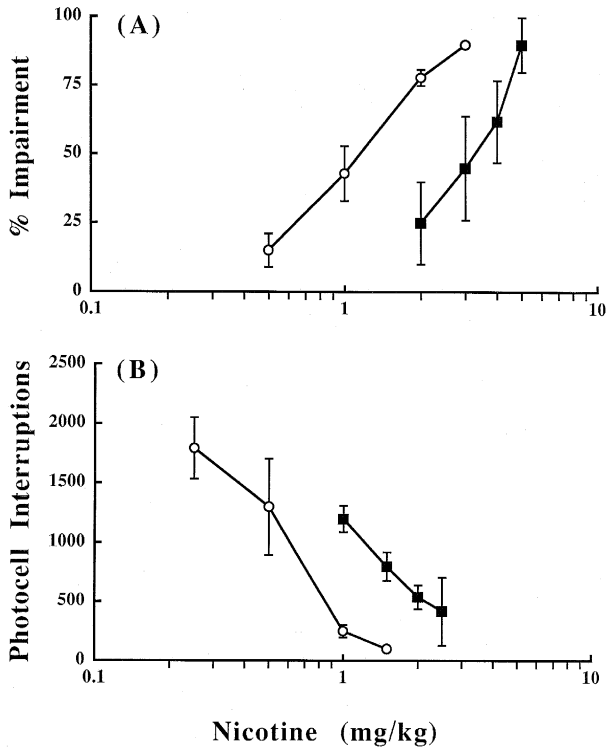

Fig. 1. Development of tolerance to nicotine-induced (A) motor impairment and (B) hypomotility in mice that were chronically injected twice daily for 10 days with either (○) saline or (■) nicotine (2 mg/kg, s.c.). The control activity for saline-treated animals is 2150 ± 350 photocell interruptions. Each point represents the mean ± S.E. of 8–12 mice.

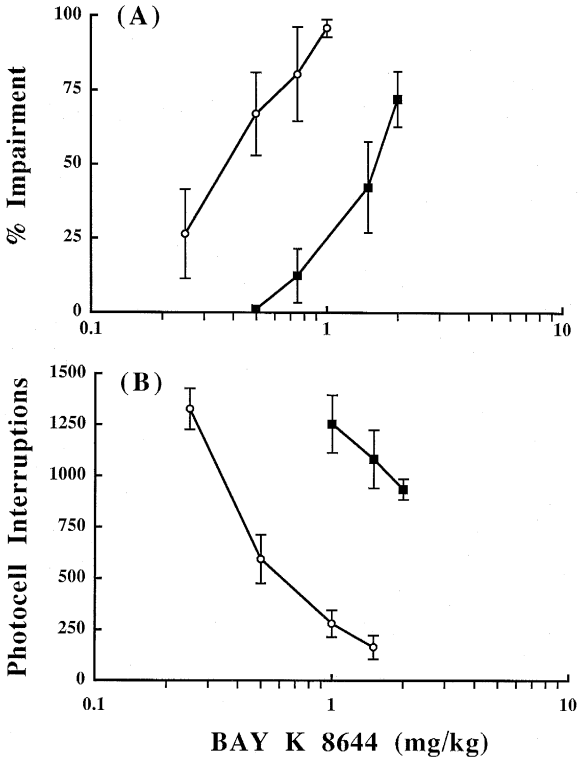

Fig. 2. Development of cross-tolerance to BAY K 8644-induced (A) motor impairment and (B) hypomotility in mice that were chronically injected twice daily for 10 days with either (○) saline or (■) nicotine (2 mg/kg, s.c.). The control activity for saline-treated animals is 1850±253 photocell interruptions. Each point represents the mean±S.E. of 8–12 mice.

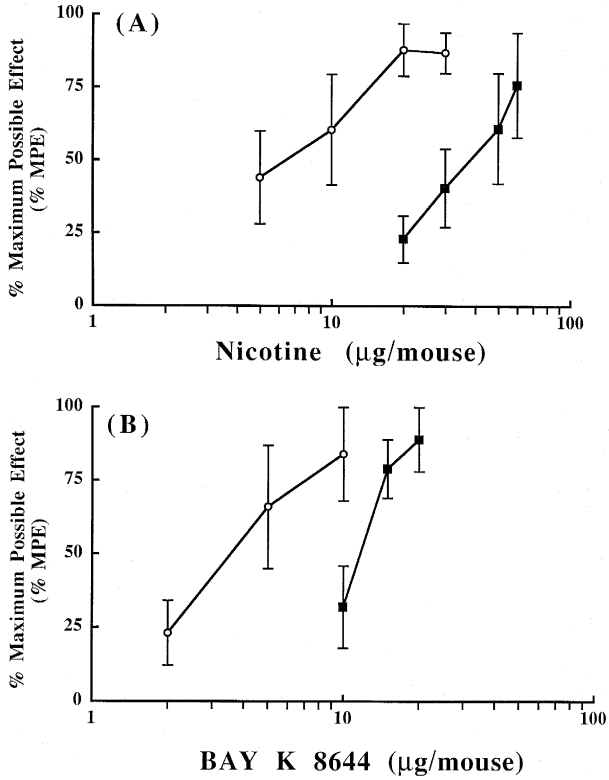

Fig. 3. Development of tolerance to the antinociceptive effect of (A) nicotine and (B) BAY K 8644 given i.t. in mice that were chronically injected twice daily for 10 days with either (○) saline or (■) nicotine (2 mg/kg, s.c.). Antinociceptive response was calculated as %MPE (maximum possible effect). Each point represents the mean±S.E. of 8–12 mice.

mice. Indeed, the dose-response curve for BAY K 8644-induced motor impairment was shifted to the right (Fig. 2A), and the ED_{50} value increased from 0.35 in saline-treated animals to 2.0 mg/kg in nicotine-tolerant mice. In addition, mice became tolerant to the effects of BAY K 8644 on locomotor activity as shown by the rightward shift of the dose-response curve after chronic nicotine (Fig. 2B). It is noteworthy to mention that minimal tolerance to the pharmacological effects of BAY K 8644 developed (10–15%) after chronic injection of BAY K 8644 at relatively high doses (8 mg/kg per day) for 8 days (data not shown).

3.2. Antinociceptive effects of Ca^{2+}-modulating drugs in nicotine-tolerant mice

To evaluate cross-tolerance at the spinal cord level between nicotine and Ca^{2+}-modulating drugs, several groups of mice chronically treated with either saline or nicotine were challenged with different doses of nicotine, BAY K 8644, thapsigargin and Ca^{2+} given i.t., and their antinociceptive action was measured. Mice became tolerant to the antinociceptive effects of these drugs as shown by the rightward shift of the dose-response curve after chronic nicotine (Figs. 3 and 4). As summarized in Table

Table 1
Effect of chronic s.c. injection of nicotine on the antinociceptive action of different Ca^{2+}-modulating drugs after intrathecal administration in mice

Drug challenge (intrathecal injection)	Chronic saline ED_{50} (±CL) μg/mouse	Chronic nicotine ED_{50} (±CL) μg/mouse	Ratio (nicotine/saline)
Nicotine	6 (2–17)	36 (22–58)	6
BAY K 8644	3.7 (1.69–8.4)	11.7 (8–16)	3
Ca^{2+}	0.32 (0.22–0.47) [a]	1.1 (0.7–1.4) [a]	3
Thapsigargin	0.83 (0.24–2.85)	20 (12.5–32)	24

Results are expressed as ED_{50} values with 95% confidence limits (±CL) and 8–12 mice were tested in each treatment group. [a] ED_{50} values are expressed in μmol/mouse.

1, ED_{50} values for nicotine increased from 6 μg/mouse in control mice to 36 μg/mouse in chronic nicotine mice. Tolerance to Ca^{2+}, thapsigargin, and BAY K 8644-induced antinociception developed also in nicotine-tolerant mice. ED_{50} values for BAY K 8644 and thapsigargin increased from 3.7 to 12 μg/mouse and 0.83 to 19.7 μg/mouse, respectively. The greatest tolerance developed to the effects of thapsigargin (Fig. 4A), a potent inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase (Thastrup et al., 1990), with a ratio close to 25-fold. It is also important to note that tolerance developed to the toxic and lethal effects of i.t. Ca^{2+} (seen usually after a dose of 1 μmol i.t.) after chronic nicotine administration.

3.3. Effects of chronic nicotine administration on [^{3}H]nitrendipine binding site in mouse brain

The binding of [^{3}H]nitrendipine to whole brain membranes from mice that received chronic nicotine or saline treatment for 10 days was investigated. Chronic nicotine injections did not alter [^{3}H]nitrendipine binding in the brain. Scatchard analysis confirmed that neither the B_{max} (112 ± 6 fmol/mg in chronic saline-injected animals versus 129 ± 8 fmol/mg in chronic nicotine-injected animals) nor the K_d (1.37 ± 0.12 nM in chronic saline-injected animals versus 1.51 ± 0.04 nM in chronic nicotine-injected animals) was altered by chronic nicotine treatment. Nicotine in vitro at 1 and 10 μM concentrations did not displace [^{3}H]nitrendipine binding.

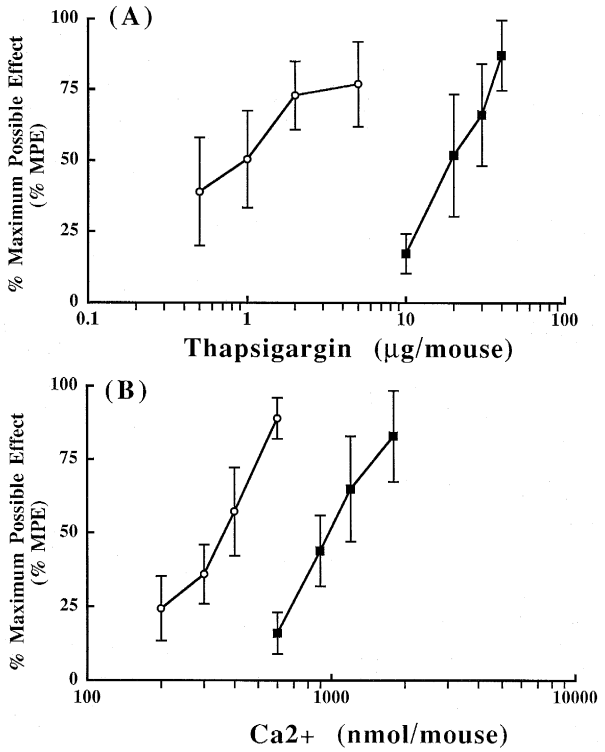

Fig. 4. Development of tolerance to the antinociceptive effect of (A) thapsigargin and (B) Ca^{2+} given i.t. in mice that were chronically injected twice daily for 10 days with either (○) saline or (■) nicotine (2 mg/kg, s.c.). Antinociceptive response was calculated as %MPE (maximum possible effect). Each point represents the mean ± S.E. of 8–12 mice.

4. Discussion

Our study using systemic and i.t. administration in whole animals to assess the role of Ca^{2+} homeostasis in nicotine tolerance, showed a cross-tolerance between nicotine and Ca^{2+}-acting drugs. The fact that a shift in the dose-response curves of these drugs was seen in nicotine-tolerant mice suggests that adapation to the effects of Ca^{2+}-acting drugs occurred after chronic administration of nicotine. However, such adaptation may not be receptor mediated. Indeed, it is reported that tolerance to nicotine-induced antinociception in rats may be influenced by learning (Epstein et al., 1989) and that the release of corticosterone could contribute to the developement to some of nicotine's effects after chronic injection of the drug (Caggiula et al., 1991, 1993). Therefore, the cross-tolerance between nicotine and Ca^{2+}-modulating drugs may have arisen from a shared reduction in response generated by environmental cues and hormonal effects. The interaction of Ca^{2+}-dependent mechanisms and processes is possible and could explain the underlying mechanisms of cross-tolerance between nicotine and Ca^{2+} drugs. It is reported that the activation of neuronal nicotinic receptors causes an influx of Ca^{2+} leading to an increase in intracellular calcium ($[Ca^{2+}]_i$) (Mulle et al., 1992). Furthermore, in cortical synaptosomes and in primary cultures of cortical neurons, application of moderate to high concentrations of nicotine (1–100 μM) was accompanied by an increase of $[Ca^{2+}]_i$ (Hillard and Graf, 1990; Lippiello, 1989). Assuming that BAY K 8644, thapsigargin and Ca^{2+} produced their functional responses by increasing intracellular Ca^{2+}, the first possibility is a development of tolerance to the rise in intracellular Ca^{2+} levels induced by these drugs. It could be hypothesized that a compensatory decrease in Ca^{2+} entry occurs after chronic exposure to nicotine and may be due to an alteration in one or more of the following membrane components that are targets for Ca^{2+}-acting drugs: (1) changes in voltage-sensitive Ca^{2+} channel affinity and numbers, in particular L-type channels, (2) changes in ATPase activities, and (3) inactivation of neuronal nicotinic receptors.

Although a change in voltage-sensitive Ca^{2+} channel affinity and numbers is a possible scenario, our binding results showed that the number and affinity of dihydropyridine-sensitive Ca^{2+} channels were not significantly changed after chronic administration. However, Ca^{2+} channels may undergo changes in their basal phosphorylation state in nicotine-tolerant animals. Such changes may not be detected by the binding assay. A decrease in

ATPase activity may also underly the cross-tolerance to Ca^{2+}-modulating drugs, in particular thapsigargin (with a tolerance ratio of 25-fold) which blocks Ca^{2+} reuptake into the microsomal Ca^{2+} pool by inhibiting the endoplasmic reticulum Ca^{2+}-ATPase (Thastrup et al., 1990). Although Marks (1987) found no significant changes in ATPase activity in different mouse brain areas after chronic infusion of nicotine, the comparison of their findings with our results must be done cautiously because nicotine was administered by injection in our studies. Recently, it has been sugested that nicotinic receptors present on chromaffin cells and neuroblastoma cell line IMR 32 contain a dihydropyridine-sensitive site (Donnelly-Roberts et al., 1995; Lopez et al., 1993). Although dihydropyridine derivatives such nifedipine and BAY K 8644 failed to displace [^{3}H]nicotine binding sites in rat brain (Damaj et al., 1993), an alteration in dihydropyridine-sensitive site may be involved in the adaptations that occur following chronic treatment with nicotine.

Another possibility is an adaptation in Ca^{2+}-dependent processes and events involved in antinociceptive or locomotive mechanisms, such as Ca^{2+}-binding proteins, Ca^{2+}-dependent protein kinase and Ca^{2+}-dependent protein phosphatase systems. Of particular interest is Ca^{2+}/calmodulin-dependent protein kinase II, a protein kinase activated by an increase in intracellular Ca^{2+} and involved in neurotransmitter release through its target synapsin I. Recently, it was reported that calmidazolium, a calmodulin inhibitor, abolished nicotine-evoked dopamine release in PC12 cells (Courtney et al., 1991). In addition, MacNicol and Schulman (1992) reported that dimethyl phenyl piperazinuim (DMPP), a nicotinic receptor agonist, activated calmodulin kinase II in PC12 cells. More recently, Ochoa and O'Shea (1994) showed an increase in the phosphorylation of an 80-kDa protein (that contains synapsin I) in rat frontal cortex synaptosomes after stimulation with nicotine. It is therefore conceivable that synapsin I, a target for the action of nicotine in the CNS, undergoes adaptation after chronic nicotine administration due to a decrease in the activation of Ca^{2+}-dependent protein kinases. In addition, chronic treatment with nicotine was reported to decrease the cortical efflux of [^{3}H]D-aspartate in freely moving guinea-pigs (Beani et al., 1991) and to induce protection of cortical neurons against glutamate cytotoxicity (Akaike et al., 1994). Our data showed that chronic nicotine administration decreased the toxic and lethal effects of Ca^{2+} when injected in the spinal cord, suggesting a protective effect of nicotine against toxcity induced by Ca^{2+}-dependent mechanisms. Other Ca^{2+}-dependent mechanisms may be involved in adaptation to nicotine. Nicotine-induced increase in intracellular Ca^{2+} would activate Ca^{2+}-sensitive adenylate cyclases which elevate cAMP levels. cAMP is reported to regulate nicotinic receptor activity and desensitization via phosphorylation of certain subunits of the receptor (Huganir and Greenhard, 1990). The activity of these kinases may decrease after chronic nicotine exposure. In addition, an adaptation to the expression of certain genes which are Ca^{2+}-dependent is also possible, especially with the recent observation that rat muscle nicotinic receptor gene expression is regulated by a Ca^{2+}-dependent signal transduction system (Walke et al., 1994). Other mechanisms such as a calmodulin kinase II regulatory phosphorylation site on the nicotinic receptor which has been described for AMPA/kainate-type glutamate receptors (Yakel et al., 1995), are possible.

Finally, functional tolerance to the different drugs described above which have different mechanisms at the cellular level, may occur downstream of the Ca^{2+} mechanisms. Although the different drugs used in this study were structurally different and tolerance developed not only after systemic but after spinal injection as well, a pharmacokinetic interaction between nicotine (given chronically) and Ca^{2+} modulating drugs still is possible.

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References

Aceto, M.D., Martin, B.R., Uwaydah, I.M., May, E.L., Harris, L.S., Izazola-Conde, C., Dewey, W.L., Vincek, W.C., 1979. Optically pure (+)-nicotine from (±)-nicotine and biological comparisons with (−)-nicotine. J. Med. Chem. 22, 174–177.

Akaike, A., Tamura, Y., Yokota, T., Shimohama, S., Kimura, J., 1994. Nicotine-induced protection of cultured cortical neurons against *N*-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. Brain Res. 644, 181–187.

Barrantes, G.E., Murphy, C.T., Westwick, J., Wonnacott, S., 1995. Nicotine increases intracellular calcium, in rat hippocampal neurons via voltage-gated calcium channels. Neurosci. Lett. 196, 101–104.

Beani, L., Tanganelli, S., Antonelli, T., Ferraro, L., Morari, M., Spalluto, P., Nordberg, A., Bianchi, C., 1991. Effect of acute and subchronic nicotine treatment on cortical efflux of [^{3}H]-D-aspartate and endogenous GABA in freely moving guinea-pigs. Br. J. Pharmacol. 102, 15–20.

Benowitz, N.L., 1992. Cigarette smoking and nicotine addiction. Med. Clin. North Am. 76, 415–437.

Bhat, R.V., Turner, S.L., Selvaag, S.R., Marks, M.J., Collins, A.C., 1991. Regulation of brain nicotinic receptors by chronic agonist infusion. J. Neurochem. 56, 1932–1939.

Caggiula, A.R., Epstein, L.H., Antelman, S.M., Saylor, S.S., Perkins, K.A., Knopf, S., Stiller, R., 1991. Conditioned tolerance to the anorectic and corticosterone-elevating effects of nicotine. Pharmacol. Biochem. Behav. 40, 53–59.

Caggiula, A., Epstein, L., Antelman, S., Saylor, S., Knopf, S., Perkins, K., Stiller, R., 1993. Acute stress or corticosterone administration reduces responsiveness to nicotine: implications for a mechanism of conditioned tolerance. Psychopharmacology 111, 499–507.

Castro, N., Albuquerque, E., 1995. α-Bungarotoxin-sensitive hippocampal nicotinic receptor channel has a high calcium permeability. Biophys. J. 68, 516–524.

Courtney, N.D., Howlett, A.C., Westfall, T.C., 1991. Regulation of

nicotine-evoked dopamine release from PC12 cells. Life Sci. 48, 1671–1678.

Damaj, M.I., Martin, B.R., 1993. Calcium agonists and antagonists of the dihydropyridine type: effect on nicotine-induced antinociception and hypomotility. Drug Alcohol Depend. 32, 73–79.

Damaj, M.I., Martin, B.R., 1996. Tolerance to the antinociceptive effect of epibatidine after acute and chronic administration in mice. Eur. J. Pharmacol. 300, 51–57.

Damaj, I., Welch, S., Martin, B., 1993. Involvement of calcium and L-type channels in nicotine-induced antinociception. J. Pharmacol. Exp. Ther. 266, 1330–1338.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Dewey, W.L., Harris, L.S., Howes, J.S., Nuite, J.A., 1970. The effect of various neurohormonal modulations on the activity of morphine and the narcotic antagonists in tail-flick and phenylquinone test. J. Pharmacol. Exp. Ther. 175, 435–442.

Donnelly-Roberts, D., Arneric, S., Sullivan, J.P., 1995. Functional modulation of human 'ganglionic-like' neuronal nicotinic acetylcholine receptors (nAChRs) by L-type calcium channel antagonists. Biochem. Biophys. Res. Commun. 213, 657–662.

Epstein, L.H., Caggiula, A.R., Stiller, R., 1989. Environment-specific tolerance to nicotine. Psychopharmacology 97, 235–237.

Hillard, C.J., Graf, W.K., 1990. Studies of the effect of nicotine on synaptosomal calcium accumulation using fura-2. NIDA Res. Monogr. 331–332.

Huganir, R.L., Greenhard, P., 1990. Regulation of neurotransmitter receptor desensitization by protein phosphorylation. Neuron 5, 555–567.

Hylden, J.L., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 67, 313–316.

Lippiello, P.M., 1989. Properties of putative nicotine receptors identified on cultured cortical neurons. In: Nordberg, A., Fuxe, K., Holmstedt, B., Sundwall, A. (Eds.), Progress in Brain Research, Vol. 79, Elsevier, Amsterdam, pp. 129–135.

Lopez, M., Fonteriz, R., Gandia, L., De la Fuente, M., Villarroya, M., Sancho, J., Garcia, A., 1993. The nicotinic acetylcholine receptor of the bovine chromaffin cell, a new target for dihydropyridines. Eur. J. Pharmacol. 247, 199–207.

MacNicol, M. and Schulman, H., 1992. Multiple Ca^{2+} signaling pathways converge on CaM kinase in PC12 cells. FEBS Lett. 304, 237–240.

Marks, M., 1987. Mouse brain ATPase activities after chronic nicotine infusion. Biochem. Pharmacol. 36, 3318–3321.

Marks, M.J., Romm, E., Gaffney, D.K., Collins, A.C., 1986. Nicotine-induced tolerance and receptor changes in four mouse strains. J. Pharmacol. Exp. Ther. 237, 809–819.

Marks, M.J., Stitzel, J.A., Collins, A.C., 1987. Influence of kinetics of nicotine administration on tolerance development and receptor levels. Pharmacol. Biochem. Behav. 27, 505–512.

Marks, M., Grady, S., Collins, A., 1993. Down-regulation of nicotinic receptor function after chronic nicotine infusion. J. Pharmacol. Exp. Ther. 266, 1268–1276.

Mulle, C., Choquet, D., Korn, H., Changeux, J.-P., 1992. Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. Neuron 8, 135–143.

Ochoa, E.L., O'Shea, S.M., 1994. Concomitant protein phosphorylation and endogenous acetylcholine release induced by nicotine: dependency on neuronal nicotinic receptors and desensitization. Cell. Mol. Neurobiol. 14, 315–340.

Pauly, J.R., Grun, E.U., Collins, A.C., 1992. Tolerance to nicotine following chronic treatment by injections: a potential role for corticosterone. Psychopharmacology 108, 33–39.

Peng, X., Gerzanich, V., Anand, R., Whiting, P.J., Lindstrom, J., 1994. Nicotine-induced increase in neuronal nicotinic receptors results from a decrease in the rate of receptor turnover. Mol. Pharmacol. 46, 523–530.

Rapier, C., Lunt, G.G., Wonnacott, S., 1988. Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. J. Neurochem. 50, 1123–1130.

Rowell, P.P., Winkler, D.L., 1984. Nicotinic stimulation of [^{3}H]acetylcholine release from mouse cerebral cortical synaptosomes. J. Neurochem. 43, 1593–1598.

Schwartz, R.D., Kellar, K.J., 1985. In vivo regulation of [^{3}H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. J. Neurochem. 45, 427–433.

Surgeon, G., 1988. The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General, U.S. Government Printing Office, Washington, DC.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacological Calculations with Computer Programs, Springer, New York, NY, p. 293.

Thastrup, O.C., Droaback, P.J., Hanley, B.K., Dawson, A.P., 1990. Thapsigargin, a tumor promotor, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}ATPase. Proc. Natl. Acad. Sci. USA 87, 2466–2470.

Vernino, S., Amador, M., Luetje, C.W., Patrick, J., Dani, J.A., 1992. Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. Neuron 8, 127–134.

Walke, W., Staple, J., Adams, L., Gnegy, M., Chahine, K., Goldman, D., 1994. Calcium-dependent regulation of rat and chick muscle nicotinic acetylcholine receptor (nAChR) gene expression. J. Biol. Chem. 269, 19447–19456.

Yakel, J.L., Vissavajjhala, P., Derkach, V.A., Brickey, D.A., Soderling, T.R., 1995. Identification of a Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in non-*N*-methyl-D-aspartate glutamate receptors. Proc. Natl. Acad. Sci. USA 92, 1376–1380.