Characterization and Modulation of Acute Tolerance to Nicotine in Mice

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ABSTRACT
Acute tolerance to the effects of nicotine is believed to play an important role in the development and maintenance of dependence to this drug. The objective of this study was to investigate and characterize the development of acute tolerance to nicotine after systemic and intrathecal administrations. Acute tolerance developed to several centrally mediated pharmacological effects of nicotine after systemic (motor coordination, body temperature, antinociception) and intrathecal (antinociception) injection of the drug. The appearance and the magnitude of acute tolerance varied depending on the response measured. Development of acute tolerance to nicotine-induced hypothermia and motor impairment was blocked after intraperitoneal pretreatment with nimodipine. Similarly, an intrathecal injection of nimodipine blocked the development of acute tolerance to nicotine-induced antinociception. On the other hand, intrathecal administration of calcium and thapsigargin enhanced the acute tolerance to nicotine-induced antinociception. Characterization of the acute tolerance to nicotine in several animal models revealed time and dose dependences that are consistent with receptor-mediated events. More importantly, acute tolerance was modulated by agents that influence cellular calcium homeostasis.

Nicotine, one of the most widely used drugs in the world, is a potent modulator of the central nervous system because of its enhancement of ion flux and neurotransmitter release. It elicits a variety of physiological and behavioral effects, including alterations in locomotor activity, cerebrovasodilatation, a discriminative stimulus cue, hypothermia, convulsions and antinociception (for a review, see Martin, 1986). Acute tolerance to the effects of nicotine develops in animals and humans (Perkins et al., 1994) and is believed to play an important role in the development and maintenance of dependence to this drug. Acute tolerance reflects short-term adaptation of the body to nicotine and may help explain typical temporal patterns of nicotine intake over the course of the day in smokers. Many smokers report that their initial cigarette of the day provides the greatest "pleasure" and that succeeding cigarettes produce less effect (Pomerleau and Pomerleau, 1992), suggesting acute tolerance development. Acute tolerance has been demonstrated for peripheral nicotinic receptors in various preparations, including the motor end plate, adrenal chromaffin cells and the PC-12 clonal line (for a review, see Ochoa et al., 1989). There also is evidence that desensitization of brain nicotinic receptors can occur. Indeed, nicotinic responses measured in synaptosomes desensitize when exposed to nicotine, as reported with nicotine-induced dopamine release (Grady et al., 1994; Rapier et al., 1988; Rowell and Hillebrand, 1994) and nicotine-stimulated 46Rb+ efflux (Marks et al., 1994).

In addition, tolerance to the behavioral effects of acute administration of nicotine has been reported. Barrass et al. (1969) demonstrated that pretreating mice with nicotine resulted in a dramatic increase in the LD50 for nicotine. Stolerman et al. (1974) observed that pretreating rats with nicotine resulted in a 2.4-fold increase in the ED50 to nicotine-induced decreases in locomotor activity. Furthermore, Dunlop et al. (1980) and Miner and Collins (1988) showed that acute tolerance to nicotine-induced seizures also occurs. In addition, development of acute tolerance to the discriminative stimulus properties of nicotine was recently reported (James et al., 1994). However, the physiological significance of acute tolerance or desensitization of the nicotinic acetylcholine receptor and modulators of desensitization is not yet fully understood. Therefore, the first objective of this study was to investigate the development of acute tolerance to nicotine in vivo after systemic and intrathecal administration. Because neuronal nicotinic receptors mediate the addictive effects of nicotine, we measured several centrally mediated pharmacological effects (motor coordination, body temperature, antinociception) after systemic injection of nicotine. We also investigated acute tolerance to the antinoc-
ceptive effect after spinal administration of nicotine in mice. To relate acute nicotine tolerance to the temporal pattern of nicotine intake in smokers, we evaluated the time course of development and dissipation of acute tolerance.

Considerable attention has been directed toward elucidation of biochemical changes associated with development of acute tolerance. Studies of peripheral-type nicotinic receptors from muscle and electric organ have provided evidence that alterations in calcium ions and CaM levels activate phosphorylation of nicotinic receptors, a process involved in receptor desensitization (Huganir and Greenhard, 1990). Nicotinic desensitization at the frog neuromuscular junction (Miledi, 1980) and to catecholamine release in adrenal chromaffin cells (Boksa and Livett, 1984) is produced by calcium-dependent mechanisms. However, relatively little is known regarding the mechanisms of nicotine desensitization in the central nervous system. Therefore, the second aim of this study was to study the mechanisms underlying acute nicotine tolerance and its modulation by calcium ions.

Materials and Methods
Animals
Male ICR mice (20-25 g) obtained from Harlan Laboratories (Indianapolis, IN) were used throughout the study. The mice were housed in groups of six and had free access to food and water.

Drugs
Nicotine was obtained from Aldrich Chemical Co. (Milwaukee, WI) and converted to the ditartrate salt as described by Aceto et al. (1979). [3H]—niacinamide (80 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Nicotine diartrate and calcium chloride were dissolved in physiological saline (0.9% sodium chloride) and administered in a total volume of 1 ml/100 g b.wt. in mice. Thapsigargin (LC Service Corp., Woburn, MA) was prepared in DMSO. All doses are expressed as the free base of the drug.

Intrathecal 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 with 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. In protocols where two or three sequential injections were required in an animal, the flicking motion of the tail could be elicited with each subsequent injection.

Pharmacological and Behavioral Assays in Mice
Motor coordination. To measure motor coordination, a wooden rod (6-cm 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 administration of nicotine. At 20 min after the injection, 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 [(1 - (test time in sec/300)) × 100]. An impairment value of 0% corresponds to the animals that remained on the rotarod for 5 min (300 sec), and impairment value of 100% corresponds to animals that fell off the rotarod within <1 sec.

Antinociception. Antinociception was measured with the tail-flick method (D'Amour and Smith, 1941) as modified by Dewey et al. (1970). A control response (2-4 sec) was determined for each animal before treatment, and a test latency was determined after drug administration. A maximum latency of 10 sec was imposed if no response occurred within that time. Antinociceptive response was calculated as percent MPE (% MPE), where (% MPE = [(test - control)/(10 - control)] × 100). An MPE value of 0% corresponds to the animals with latencies that did not change from their preinjection latencies, and MPE value of 100% corresponds to animals with latencies that reached a maximum level of 10 sec in the tail-flick test. Groups of 6 to 12 animals were used for each dose and for each treatment. The mice were tested 5 min after subcutaneous administration for the nicotine dose-response evaluation. For the intrathecal experiments, mice were tested 5 min after injection. The acute tolerance studies involved three intrathecal injections in the following sequence: a first injection of either calcium, nimodipine, thapsigargin or vehicle followed by a second injection of either nicotine or saline 10 (for nimodipine) or 20 min (for calcium and thapsigargin) later and finally a third injection of nicotine or saline 10 min later. Mice were tested 5 min after the third intrathecal administration.

Body temperature. Rectal temperature was determined with a thermistor probe (inserted 24 mm) and a digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Readings were taken just before and at 30 min after the subcutaneous injection of either saline or nicotine. The flick method of D'Amour and Smith (1941) was used to determine the effect of nicotine on rectal temperature before and after treatment was calculated for each mouse. Results were expressed as ΔT, which represents the difference in rectal temperature between preinjection and postinjection values. The daily ambient temperature of the laboratory varied from 21°C to 24°C.

To determine acute tolerance to nicotine-induced motor impairment, antinociception and hypothermia, mice were pretreated subcutaneously with different doses of nicotine at different times before a second subcutaneous injection of nicotine. To evaluate the degree of shift in the dose-response curves of nicotine, mice were pretreated with a dose of nicotine that caused a comparable degree of tolerance (84%) in all of the pharmacological tests performed.

Statistical Analysis
Data were analyzed statistically with analysis of variance followed by the Fisher's PLSD multiple-comparison test. The null hypothesis was rejected at the .05 level. ED50 values with 95% 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). The effects of drugs on rectal temperature and locomotor activity were calculated from double reciprocal analysis (1/effect vs. 1/dose) to yield a theoretical maximum effect (efficacy), as described by Tallarida and Murray (1987). The ED50 values were determined by calculating the functional response for each drug dose (based on the maximum effect being 1.0), converting the data to probit values and determining the unweighted least-squares linear regression for the log-dose vs. probit as described by Tallarida and Murray (1987). Tests for parallelism and dose ratios were calculated according to the method of Tallarida and Murray (1987). A dose ratio with a lower CL of >1 or a dose ratio with an upper CL of <1 was considered the represent a significant shift in the dose-response curve (Tallarida and Murray, 1987).

Results
Acute tolerance to nicotine-induced antinociception after subcutaneous and intrathecal administrations. Initial studies were conducted to determine the pretreatment time and the dose of nicotine necessary to induce acute tolerance to the antinociceptive effect of nicotine after subcutaneous and intrathecal injections. Experiments were conducted to assay the time course for nicotine-induced acute tolerance in the tail-flick test after subcutaneous administration by pretreating the mice with 4 mg/kg nicotine (a dose that produces maximal antinociceptive effects) and then challenging them with nicotine at 2 mg/kg subcutaneously. As seen from the
results presented in figure 1A, maximum acute tolerance occurred between 0.5 and 1 hr after nicotine pretreatment. Recovery of the pretolerance response was achieved after 6 hr.

To examine the dose-response relationship of acute tolerance experiments were performed in which the effect was assessed of subcutaneous pretreatment with different doses of nicotine. Pretreatment with nicotine at doses of ≥3 mg/kg caused a complete loss of the effect of nicotine administered 1 hr later (fig. 1B). An analysis of the dose-response relationship showed that doses of 1 and 4 mg/kg nicotine decreased the effects of a 4 mg/kg dose of nicotine by 50% and 84%, respectively, in the tail-flick test after subcutaneous administration. Therefore, a pretreatment time of 1 hr and a subcutaneous dose of 4 mg/kg were selected for further studies.

A single pretreatment with nicotine (4 mg/kg subcutaneously) resulted in a 3.0-fold rightward shift in the potency of nicotine in the tail-flick test (fig. 1C). The ED₅₀ values (and 95% CL) for nicotine-induced antinociception after pretreatment with saline and nicotine (4 mg/kg subcutaneously) were 1.1 (0.6–1.4) and 3.4 (2.4–4.7) mg/kg, respectively (table 1). The ED₅₀ values represent an increase in tail-flick latencies from 3.1 to 5.3 sec and from 2.8 to 5.1 sec in saline-pretreated and nicotine-pretreated mice, respectively. Calculation of the potency ratio (3.2) and 95% CL (2.1–5.1) from the log dose-response lines indicated that this degree of acute tolerance was statistically significant.

In mice pretreated intrathecally with 1 µg nicotine/animal and then challenged at different times with nicotine (20 µg/animal intrathecally), acute tolerance to nicotine-induced antinociception occurred very rapidly after intrathecal administration, with a maximum between 5 and 10 min after injection (fig. 2A). Although tail-flick responsiveness appeared to be depressed at 2 hr, this time point was not statistically different from 0 hr. A complete loss of the effect of nicotine was seen after pretreatment with subactive intrathecal doses of 0.5 and 1 µg/animal of nicotine (fig. 2B). An analysis of the dose-response relationship showed that doses of 0.05 and 1 µg/animal of nicotine decreased the effects of a 20 µg/animal dose of nicotine by 50% and 84%, respectively, in the tail-flick test after intrathecal administration. Therefore, a pretreatment time of 10 min and an intrathecal dose of 1 µg/animal were selected for further studies.

Similar to the subcutaneous injection, pretreatment with nicotine (1 µg/animal intrathecally) resulted in a 2.5-fold rightward shift in potency of nicotine in the tail-flick procedure (fig. 2C). The ED₅₀ values (and 95% CL) for nicotine-induced antinociception after pretreatment with saline and nicotine (1 µg/animal intrathecally) were 11.7 (7.8–17.6) and 29.5 (20.4–42.4) µg/animal, respectively (table 1). Calculation of the potency ratio (2.5) and 95% CL (2.0–3.2) from the log dose-response lines indicated that this degree of acute tolerance was statistically significant.

Acute tolerance to nicotine-induced hypothermia and motor impairment. Pretreatment subcutaneously with high doses of nicotine produced a pronounced acute tolerance to motor effects in mice. As illustrated in figure 3A, maximum acute tolerance occurred 3 to 6 hr after the injection. The amount of acute tolerance to these effects, as in antinociception, was found to be dose dependent. Pretreatment doses of 2 and 4 mg/kg nicotine produced 50% and 84% motor impairment after subcutaneous injection, respectively (fig. 3B). Therefore, a pretreatment time of 3 hr and a subcutaneous dose of 4 mg/kg were selected for further studies.

A single pretreatment with nicotine (4 mg/kg subcutaneously) resulted in a significant shift to the right of the nicotine dose-response curve in the motor impairment test (fig. 3C). The ED₅₀ values (and 95% CL) for nicotine-induced
motor impairment after pretreatment with saline and nicotine (4 mg/kg subcutaneously) were 1.0 (0.5—2.2) and 5.4 (2.5—11.0) mg/kg, respectively (table 1). The ED$_{50}$ values represent a decrease in the amount of time remaining on the rod from 285 to 140 sec and from 285 to 148 sec in saline- and nicotine-pretreated mice, respectively. A potency ratio of 2.9 (95% CL = 2.0—3.7) indicated that this degree of acute tolerance was statistically significant.

Acute tolerance to nicotine-induced hypothermia in mice occurred with a maximum between 2 to 4 hr after subcutaneous injection (fig. 4A). However, the estimated dose that causes 84% acute tolerance was calculated to be 9.5 mg/kg, a lethal dose of nicotine (fig. 4B). Therefore, a pretreatment dose of 4 mg/kg (which causes 50% acute tolerance in hypothermia) and a time of 3 hr were used in these experiments. Surprisingly, a single pretreatment with nicotine (4 mg/kg subcutaneously) induced a larger shift in the dose-response curve for nicotine-induced hypothermia (fig. 4C) than in nicotine-induced motor impairment. The ED$_{50}$ values (and 95% CL) for nicotine-induced hypothermia after pretreatment with saline and nicotine (4 mg/kg subcutaneously) were 1.0 (0.5—2.2) and 5.4 (2.5—11.0) mg/kg, respectively (table 1).

Effect of nimodipine on acute tolerance induced by subcutaneous nicotine. The effect of nimodipine, a calcium channel antagonist, on acute tolerance induced by subcutaneous nicotine pretreatment was investigated in the different pharmacological tests. Pretreatment with 20 mg/kg nimodipine at 15 min before the first dose of nicotine significantly attenuated acute tolerance to nicotine-induced antinociception [potency ratio of 1.39 (1.08—1.8, 95% CL)] (fig. 5A). However, at that dose, nimodipine significantly reduced the antinociceptive effects of nicotine in mice (nicotine (2 mg/kg subcutaneously) = 82 ± 16% MPE; nimodipine plus nicotine (2 mg/kg subcutaneously) = 22 ± 10% MPE). Lower doses of nimodipine (5 and 10 mg/kg) did not reduce significantly acute tolerance to nicotine (data not shown). Development of acute tolerance to nicotine-induced motor impairment and hypothermia was blocked after pretreatment with nimodipine (5 mg/kg intraperitoneally) (fig. 5, B and C). Indeed, as presented in table 2, the ED$_{50}$ values (and 95% CL) for the
Fig. 5. Effect of nimodipine on acute tolerance induced by subcutaneous nicotine to the (A) antinociceptive, (B) motor and (C) hypothermic effects in mice. Mice received an intraperitoneal injection of nimodipine (20 mg/kg for the tail-flick test and 5 mg/kg for the other two tests), followed by a subcutaneous injection of nicotine (4 mg/kg). At a specified time after the first nicotine injection (1 hr for the tail-flick test and 3 hr for the other tests), animals were challenged with nicotine, and dose-response curves were generated in the different tests. Each point represents the mean ± S.E. of 6 to 12 mice. The treatments were nimodipine/nicotine/nicotine, vehicle/nicotine/saline and vehicle/nicotine/nicotine.

TABLE 2
Effect of nimodipine on acute tolerance induced by subcutaneous nicotine in mice

<table>
<thead>
<tr>
<th>Pharmacological effect</th>
<th>Vehicle*</th>
<th>Nicotine (4 mg/kg)</th>
<th>Nimodipine (5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antinociception</td>
<td>1.1 (0.6—1.9)</td>
<td>3.5 (2.5—4.8)</td>
<td>2.0 (1.6—2.7)*</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>1.2 (0.4—2.3)</td>
<td>5.0 (2.8—1.2)</td>
<td>1.4 (0.7—3.0)</td>
</tr>
<tr>
<td>Motor impairment</td>
<td>1.1 (0.4—2.5)</td>
<td>3.4 (2.8—5.6)</td>
<td>1.4 (0.7—2.7)</td>
</tr>
</tbody>
</table>

* Mice were pretreated with a dose of 20 mg/kg nimodipine.

Fig. 4. Acute tolerance to nicotine-induced hypothermia after subcutaneous administration in mice. A, Time course of acute tolerance. Mice were pretreated with 4 mg/kg nicotine subcutaneously and then challenged at different times with 2 mg/kg nicotine subcutaneously. Time 0 hr, vehicle at 3 hr before treatment before 2 mg/kg nicotine control. •, Statistically different from saline-nicotine (2 mg/kg subcutaneously) at P < .05. B, Dose-response relationship of acute tolerance. Mice were pretreated with different doses of nicotine and then challenged 3 hr later with a dose of 2 mg/kg nicotine. *, Statistically different from saline pretreatment (dose of 0 mg/kg nicotine) at P < .05. C, Effect of nicotine pretreatment (4 mg/kg subcutaneously) on hypothermic effect of nicotine. Mice were challenged 3 hr after the first injection with either saline or nicotine. Δt. Difference in rectal temperature between preinjection and postinjection values. Each point represents the mean ± S.E. of 6 to 12 mice.

hypothermic and motor impairment effects of nicotine were 1.4 (0.7—3.0) and 1.4 (0.7—2.7) mg/kg after nimodipine administration, respectively. At a dose of 5 mg/kg, nimodipine did not block the agonists effects of nicotine in either test. In the case of nicotine-induced hypothermia, nimodipine did not significantly reduce effects of nicotine on body temperature (nicotine, −4.2 ± 0.2°C; nimodipine+nicotine, −3.8 ± 0.2°C). Similar results were recorded for motor impairment where nimodipine did not attenuate the motor effects of nicotine (nicotine, 77.3 ± 4.0% impairment; nimodipine plus nicotine, 77.3 ± 3.2% impairment).

Effect of calcium-modulating drugs on acute tolerance to nicotine at the spinal level. The intrathecal administration of calcium (18 μg/mouse) 20 min before the first dose of nicotine (1 μg/mouse) enhanced the acute tolerance to nicotine-induced antinociception in the tail-flick test. Calcium shifted the nicotine dose-response curve in mice pretreated intrathecal with nicotine (1 μg/animal) further to the right (fig. 6) [potency ratio of 1.6 (1.3—2.0 95% CL)], and ED_{50} values (and 95% CL) increased from 30 (21—42) μg/animal in nicotine-tolerant mice to 50 (42—62) μg/animal in calcium-treated mice. Calcium by itself did not produce a significant effect on the tail-flick test (16 ± 5% MPE) at the dose and time used. In addition, thapsigargin, the potent inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase (Thastrup et al., 1990), at a dose of 0.64 μg/mouse enhanced the acute tolerance to nicotine. Indeed, a dose of nicotine (50 μg intrathecal) that produces 86% MPE in tolerant mice was inactive after
pretreatment with 0.64 µg thapsigargin (fig. 7A). Administered alone, thapsigargin at the doses tested did not affect basal thresholds, nor did it potentiate the effect of a low dose of nicotine (1 µg/animal intrathecally) in the tail-flick test (thapsigargin/saline/saline, 3 ± 1% MPE; thapsigargin/nicotine 1 µg/saline, 13 ± 11% MPE). On the other hand, intrathecal administration of nimodipine at 0.5 and 2 µg/animal blocked the development of acute tolerance to nicotine-induced antinociception (nicotine challenge at 20 µg/animal) in the tail-flick test (fig. 7B). At the dose and time used, nimodipine did not affect the antinociceptive effect of a 20 µg/animal dose of nicotine (nimodipine 2 µg/saline/nicotine 20 µg, 76 ± 22% MPE; DMSO/saline/nicotine 20 µg, 84 ± 16% MPE).

**Discussion**

The results of this study demonstrate that exposure of mice to a single dose of nicotine causes a pronounced attenuation of the effects of a subsequent dose of nicotine. These results are consistent with the limited number of studies examining acute tolerance to nicotine in vivo (James et al., 1994; Stolerman et al., 1973, 1974; Tripathi et al., 1982). They verify that the appearance and magnitude of acute tolerance vary depending on the response measured.

Although the same subcutaneous pretreatment dose (4 mg/kg) of nicotine was used in all the tests, differences in the time course and degree of acute tolerance were observed. Maximum tolerance was more rapidly obtained for the antinociceptive effect than for the thermal and motor effects (1 hr vs. 4 hr). However, a greater degree of acute tolerance occurred to nicotine-induced hypothermia.

Differences in the time course of acute tolerance were observed between the different measures. Dissipation time for acute tolerance to nicotine-induced antinociception (between 3 and 6 hr) was shorter than that observed with motor effects (between 8 and 24 hr). In a Y-maze, Stolerman et al. (1973) showed that acute tolerance develops after a single dose of nicotine (1 mg/kg), with a maximal tolerance after 2 hr and recovery by 8 hr. In another study, Hakan and Kair (1991) demonstrated that after a pretreatment dose of 1.8 mg/kg nicotine, the behavioral response to nicotine (increase in locomotor activity) was attenuated for >5 hr. In addition, Tripathi et al. (1982) showed that acute tolerance to nicotine-induced antinociception in rats was at a maximum up to 4 hr after nicotine pretreatment and lasted for 18 hr. However, no significant acute tolerance to nicotine in mice was observed by these investigators. The difference between that study and the current study could be explained by the fact that Tripathi et al. (1982) pretreated mice with low doses of nicotine insufficient to induce acute tolerance. Human studies also suggest a different time course for the dissipation of acute tolerance to nicotine. Porchet et al. (1992) estimated a half-life of 35 min for dissipation of acute tolerance to cardiovascular effects of intravenous nicotine after a single pretreatment dosing. Recently, Perkins et al. (1995) observed that acute tolerance to the subjective and cardiovascular effects of nicotine did not dissipate within 2 hr. The differences in appearance, dissipation and magnitude of acute tolerance to the different nicotinic responses observed in our study suggest the involvement of different nicotinic receptor...
Subtypes (with different desensitizing properties) and/or neural pathways in mediating these effects.

In contrast to the results obtained with subcutaneous injection, acute tolerance to the effects of nicotine at the spinal level seems to develop after pretreatment with subactive doses of intrathecal nicotine. Indeed, the dose of nicotine able to produce 50% acute tolerance was 0.05 μg/animal, a dose well below that producing antinociception. Furthermore, a distinct pattern of development and dissipation of acute tolerance was seen after intrathecal administration. Further experiments with central administration (intracerebroventricular) of nicotine could clarify whether spinal nicotinic responses exhibit different acute tolerance profiles than do those in the brain. It is not unreasonable to suspect differences between spinal and supraspinal profiles as Khan et al. (1994) demonstrated that the spinal nicotinic receptors differ from those in ganglia and those characterized in brain.

Although no direct evidence is presented, central receptor-mediated mechanisms are probably involved in the development of acute tolerance to nicotine as the different effects are totally (antinociception and motor impairment) or partially (hypothermia) mediated by central nicotinic receptors (Damaj et al., 1995; Sahley and Berntson, 1979; Tripathi et al., 1982), and the degree of tolerance is dose dependent.

Desensitization of central nicotinic receptors is a likely mechanism by which acute tolerance develops. Electrophysiological as well as ligand binding kinetic studies suggest that desensitization occurs by at least two processes, i.e., a fast process with rates in the millisecond range and a slower process in the range of seconds (Feltz and Trautmann, 1982; Sakmann et al., 1980; Walker et al., 1982). Although the results of our studies would only be expected to assess the slow desensitization process, it appears from our findings that both the development of and the recovery from desensitization are somewhat slower in vivo compared with studies using patch-clamp, stop-flow or binding studies. Long-lasting desensitization of nicotinic currents has been noted previously in PC12 cells (Boyd and Leeman, 1987; Simasko et al., 1996), neurotransmitter synaptosomal release experiments (Rowell and Hillebrand, 1994) and in vivo studies (Miner and Collins, 1988). In addition, distribution and metabolic factors and neurophysiological factors other than desensitization could also play a role in the development of acute tolerance to nicotine (James et al., 1994).

It is possible that receptor inactivation produced by active pretreatment doses of nicotine plays a role in the acute tolerance process. It has been demonstrated that several processes subsequent to nicotinic receptor activation in the periphery, such as Na⁺ and Ca²⁺ channel opening, can also undergo desensitization (Marley, 1988). Desensitization was described for nicotine-induced norepinephrine release (a calcium-dependent process) in the rat hypothalasum in response to a second injection of nicotine (Sharp and Matta, 1989).

Desensitization of nicotinic receptors, in particular, those in muscle, can be modulated by exogenous and endogenous substances. Nicotinic receptors can be phosphorylated by protein kinases A and C and by tyrosine protein kinases (Huganir and Greenhard, 1990). Neuropeptides like calcitonin gene-related peptide and substance P directly and indirectly affect (via a second messenger system) desensitization of the muscle nicotinic receptor (Ochoa et al., 1989). Intracellular calcium is another modulator of nicotinic receptor desensitization. A number of early electrophysiological studies with muscle and peripheral nicotinic receptors indicated that desensitization of the nicotinic receptor increased with the concentration of calcium (Miledi, 1980; Parsons et al., 1973). These effects of calcium are apparently due to the direct action of calcium at the intracellular site of the receptor itself (Miledi, 1980).

Our results showed in vivo that acute tolerance to nicotine is blocked by nimodipine, a calcium channel antagonist that is consistent with the above-cited electrophysiological studies. The mechanisms of the effects of nicotine on acute tolerance are still unclear. A specific interaction between nimodipine and neuronal nicotinic receptors as described with calcium channel blockers (verapamil, methoxyverapamil and nicardipine) and nicotinic receptors in the neuromuscular junction (Seillick, 1983) is possible. However, these calcium channel blockers lack affinity for the [3H]nicotine binding sites in the brain (Damaj et al., 1993). In addition, our previous studies indicate that nimodipine does not modulate the effects of nicotine in a variety of tests, except for a partial attenuation of nicotine-induced antinociception after subcutaneous administration (Damaj and Martin, 1993). Although a direct interaction with neuronal nicotinic receptors cannot be totally ruled out, the modulatory effect of nimodipine may be mediated by a decrease in calcium influxes through voltage-dependent calcium channels.

Further evidence for a role of calcium in the acute tolerance of the spinal nicotinic responses was provided by calcium and thapsigargin, which increase intracellular calcium through voltage-gated calcium channels and through intracellular inositol triphosphate receptors, respectively. Indeed, administration of calcium and thapsigargin significantly enhanced the degree of acute tolerance. The effect of these calcium-modulating drugs on the dissipation time is not known because the effects of calcium pretreatment on the time course of acute tolerance were not investigated. On the other hand, nimodipine could block acute tolerance at the spinal level by decreasing intracellular calcium influxes.

In conclusion, the results of this study have shown that prior administration of nicotine produces a pronounced attenuation in the ability of a subsequent nicotine challenge to produce a pharmacological effect. Characterization of this acute tolerance in several animal models revealed time and dose dependencies that are consistent with receptor-mediated events. More important, this acute tolerance can be modulated by agents that influence cellular calcium homeostasis. Modulation of the function of the central nicotinic receptors by desensitization may have important implications, particularly with respect to nicotine dependence. Clearly, future research on rapid tolerance and its modulation may be the key to a better understanding of many physiological and pathological processes taking place within the central and peripheral nervous systems.

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References


References

Acute Tolerance to Nicotine


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