Influence of Gender and Sex Hormones on Nicotine Acute Pharmacological Effects in Mice

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ABSTRACT

The present study conducted a comprehensive examination of the putative sex differences in the potency of nicotine between male and female ICR mice using several pharmacological and behavioral tests. Among the responses to nicotine where significant sex differences were observed are the antinociceptive and the anxiolytic effects of nicotine. Female mice were found less sensitive to the acute effects of nicotine in these tests after s.c. administration. Similar gender differences were found after i.t. injection. Influence of gonadal hormones could underlie sex differences observed in our studies. Indeed, our data clearly indicate that sex hormones can modulate the effects of nicotine and nicotinic receptors in a differential manner. Progesterone and 17β-estradiol were found to block nicotine’s antinociception in mice. Testosterone failed to do so. In addition, progesterone and 17β-estradiol blocked nicotine activation of α4β2 neuronal acetylcholine nicotinic receptors expressed in oocytes. Our findings contribute to our search for receptor mechanisms in drug dependence and in the discovery of better pharmacological agents for nicotine dependence.

In spite of heightened education and prevention strategies, cigarette smoking remains a major health risk. Nicotine is believed to be the primary reason that people consume tobacco products. Indeed, substantial evidence now shows that nicotine is the addictive substance found in tobacco. In 1974, 31% of U.S. women and 43% of U.S. men were tobacco smokers. Current estimates indicate that the difference between men and women has narrowed considerably to a comparable rate (21–25%) (CDC, 1998). Furthermore, poorer outcome of women in smoking cessation trials, especially those involving nicotine replacement, has also been reported. In other words, nicotine replacement is less effective for smoking cessation than in men. There are several potential causes for this shift toward more women smokers. There are several possible explanations for this trend. Factors such as women’s greater concern about weight gain, greater difficulty with negative mood (and higher prevalence of affective disorders), and greater need for social support to quit smoking could be of influence. Reduced availability of social support for cessation in women and greater impact of advertising on promoting smoking in women versus men may also have an influence. Broad cultural influences also have been advanced as an explanation for sex differences in smoking prevalence. Although cultural factors are clearly important in explaining the historical differences between the smoking patterns of American men and women earlier this century and in non-Western countries, they do not seem to be a likely explanation. Indeed, cultural restrictions in smoking behavior specific to women in Western societies are no longer in existence. One obvious possibility is the involvement of biological differences between the sexes might contribute to this gender difference.

Animal studies have found that male and female rodents have different sensitivities to the effects of nicotine. For example, female rats are less sensitive than male rats to the discriminative stimulus effects of nicotine (Schechter and Rosecrans, 1971), consistent with findings in humans (Perkins et al., 1999). Female mice were less sensitive to nicotine-induced suppression of Y-maze activity (Hatchell and Collins, 1980) and increase in active avoidance learning (Yilmaz et al., 1997). In addition, sex differences in receptor up-regulation after chronic exposure to nicotine was also reported with only male rats showing up-regulation of brain [3H]cytisine binding sites (Koylu et al., 1997). Conversely, female rats were more sensitive to nicotine’s effects on pre-pulse inhibition (Faraday et al., 1999) and food intake (Grunberg et al., 1984). Female rats were also more sensitive to nicotine-induced antinociception compared with males in acute and chronic pain models (Craft and Milholland, 1998; Chiari et al., 1999; Lavand’homme and Eisenach, 1999). In addition, sensitization to nicotine-induced increase in locomotor activity was found to be greater in female rats after

**ABBREVIATIONS:** nAChR, acetylcholine nicotinic receptor; %MPE, percentage maximum possible effect; i.t., intrathecal; CL, confidence limit; AD50, antagonist dose 50%; ACh, acetylcholine.

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chronic i.v. administration of nicotine (Booze et al., 1999), but not after chronic s.c. injection (Kanyt et al., 1999). Such complexity and variability in the response of male and female rodents to nicotine may be due to differences in the dose and elimination profile of nicotine, age and species of the test animal, route or duration of administration, time of evaluation, or the behavioral task used.

Another biological factor that could underlie sex differences is the influence of gonadal hormones. Levels of sex hormones change through the menstrual (human) or estrous (rodent) cycle. Human studies examining the effects of the phase of the menstrual cycle on cigarette smoking and on the psychoactive actions of nicotine are conflicting. However, most studies report that menstrual cycle phase may influence self-reported withdrawal symptoms in women (Benowitz and Hatsukami, 1998). Animal studies suggest that ovarian hormones could influence nicotine acute effects and reinforcement. Dluzen and Anderson (1997) reported that estrogen treatment to ovariectomized female rats increased the in vitro nicotine-induced release of dopamine from striatal slices, whereas the opposite effect was seen in castrated males.

Therefore, the aim of the present study was to conduct a comprehensive examination of the putative sex differences in the potency of nicotine between male and female mice using several pharmacological and behavioral tests. For that, the potency of nicotine in various pharmacological effects measures (antinociception, hyperthermia, seizures, and motor activity, plus-maze activity) in animals was examined after systemic and intrathecal administration. A wide range of nicotinic effects is important to consider because it is believed that various nicotinic receptor subtypes mediate different pharmacological effects of nicotine. Because gonadal hormones may also mediate such sex differences, the effects of testosterone, 17β-estradiol, and progesterone on nicotine’s effects were examined. The in vivo effect of hormones was correlated with in vitro studies using the oocyte expression system. For that, the effect of sex hormones on the functional activity of the neuronal nAChR $\alpha_4\beta_2$ (the major nicotinic receptor subtype in the brain) expressed receptors was studied.

Materials and Methods

Animals

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

Drugs

(−)-Nicotine was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) and converted to the ditartrate salt as described by Aceto et al. (1979). (−)-Epibatidine (hemi oxalate salt) was supplied by Dr. S. Fletcher (Merck Sharp and Dohme & Co., Essex, UK). Mecamylamine hydrochloride was supplied as a gift from Merck, Sharp and Dohme & Co. (West Point, PA). Testosterone, 17β-estradiol, and progesterone-water-soluble (cyclodextrin-encapsulated) and oil-soluble (testosterone decanoate) forms were purchased from Sigma Chemical Co. (St. Louis, MO); neostigmine was purchased from Research Biochemicals International (Natick, MA). Oil-soluble hormones were diluted in sesame oil (Sigma Chemical Co.). All other drugs were dissolved in physiological saline (0.9% sodium chloride) and given in a total volume of 1 ml/100 g of body weight for s.c. injections. All doses are expressed as the free base of the drug.

Intrathecal Injections

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 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.

Antinociceptive Tests

Tail-Flick Test. Antinociception was assessed by the tail-flick method of D’Amour and Smith (1941) as modified by Dewey et al. (1970). A control response (2–4 s) was determined for each mouse before treatment, and a test latency was determined after drug administration. To minimize tissue damage, a maximum latency of 10 s was imposed. Antinociceptive response was calculated as percentage maximum possible effect (%MPE), where $%MPE = [(test\ -\ control)/(10\ -\ control)] \times 100$. Groups of 8 to 12 animals were used for each dose and for each treatment. The mice were tested 5 min after either s.c. or i.t. injections of nicotine.

Hot-Plate Test. The method is a modification of that described by Eddy and Leimbach (1953) and Atwell and Jacobson (1978). Mice were placed into a 10-cm-wide glass cylinder on a hot-plate (Thermojust Apparatus, Richmond, VA) maintained at 55.0°C. Two control latencies at least 10 min apart were determined for each mouse. The normal latency (reaction time) was 6 to 10 s. Antinociceptive response was calculated as %MPE, where $%MPE = [(test\ -\ control)/(40\ -\ control)] \times 100$. The reaction time was scored when the animal jumped or licked its paws. Eight mice per dose were injected s.c. with nicotine and tested 5 min after injection.

The effects of testosterone, 17β-estradiol, and progesterone on nicotine’s antinociceptive effects were examined. Female (17β-estradiol and progesterone) and male (testosterone) mice were pretreated with different doses of hormones given i.p. and then challenged with s.c. nicotine at different times. After determining the time-course effect of hormones, the potency of these hormones in blocking nicotine-induced antinociception was performed at the maximum time.

Behavioral Testing

Locomotor Activity. Mice were placed into individual Omnitech photocell activity cages (28 × 18.5 cm) 5 min after s.c. administration of either 0.9% saline or nicotine. Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 10 min. Data are expressed as number of photocell interruptions.

Body Temperature. Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Readings were taken just before and at 30 min after the s.c. injection of either saline or nicotine. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory was 21–24°C from day to day.

Seizure Activity. After a s.c. injection of nicotine at a dose of 9 mg/kg, each animal was placed in a 30–30 cm Plexiglas cage and observed for 5 min. Whether a clonic seizure occurred within a 5-min time period was noted for each animal after s.c. administration of different drugs. This amount of time was chosen because seizures occur very quickly after nicotine administration. Results are expressed as percentage seizure.

Elevated Plus-Maze. An elevated plus-maze, prepared with gray Plexiglas, consisted of two open arms (23 × 6.0 cm) and two enclosed arms (23 × 6 × 15 cm) that extended from a central platform. It was mounted on a base raised 60 cm above the floor. Fluorescent lights
(350-lux intensity) located in the ceiling of the room provided the only source of light to the apparatus. The animals were placed in the center of the maze and the following variables were scored: 1) time spent in the open arms, 2) time spent in the closed arms, and 3) total number of crossings between arms. These variables are automatically recorded by a photocell beam system. The test lasted 5 min and the apparatus was thoroughly cleaned after removal of each animal. Readings were taken 15 min after the s.c. injection of either saline or nicotine. Anxiolytic response was calculated as the percentage of time spent in the open arm, where % time = [(test – 300 s) × 100].

Oocyte Expression Studies

Oocyte Preparation. Oocytes preparation was performed according to the method of Mirshahi and Woodward (1995) with minor modifications. Briefly, oocytes were isolated from female adult oocyte-positive Xenopus laevis frogs. Frogs were anesthetized in a 0.2% 3-aminobenzoic acid ethyl ester solution (Sigma Chemical Co.) for 30 min and a fraction of the ovarian lobes was removed. The eggs were rinsed in Ca²⁺-free ND96 solution, treated with collagenase type IA (Sigma Chemical Co.) for 1 h to remove the follicle layer, and then rinsed again. Healthy stage V-VI oocytes were selected and maintained for up to 14 days after surgery in 0.5× L-15 media (Sigma Chemical Co.).

mRNA Preparation and Microinjection. α4 and β2 rat subunit cDNA contained within a pcDNANeo vector were kindly supplied by Dr. James Patrick (Baylor College of Medicine, Houston, TX). The template was linearized downstream of coding sequence and mRNA was synthesized using an in vitro transcription kit from Ambion (Austin, TX). The quantity and quality of message were determined via optical density (spectrophotometer; Beckman Instruments Inc., Chaumburg, IL) and denaturing formaldehyde gel analysis. Oocytes were injected with either 51 ng (41 nl) of α4 and β2 mRNA mixed in a 1:1 ratio using a Variable Nanoject (Drummond Scientific Co., Broomall, PA). Oocytes were incubated in 0.5× L-15 media IA supplemented with penicillin, streptomycin, and gentamicin for 4 to 6 days at 19°C before recording.

Electrophysiological Recordings. Oocytes were placed within a Plexiglas chamber (total volume 0.2 ml) and continually perfused (10 ml/min) with buffer consisting of 115 mM NaCl, 1.8 mM CaCl₂, 2.5 mM KCl, 1.0 µM atropine, and 10.0 mM HEPES at pH 7.2. Oocytes were impaled with two microelectrodes containing 3 M KCl (0.2–3 MΩ) and voltage-clamped at −70 mV using an Axon Ge-neclamp amplifier (Axon Instruments Inc., Foster City, CA). Oocytes were stimulated for 10 s with various concentrations of acetylcholine using a six-port injection valve. Except where noted, applications were separated by 5-min periods of washout. Currents were filtered at 10 Hz and collected by a Macintosh Centris 650 with a 16-bit analog digital interface board, and data were analyzed using Pulse Control voltage-clamp software running under the Igor Pro graphic platform (Wavemetrics, Lake Oswego, OR). Water-soluble forms of hormones were applied at different concentrations and concentration-response curves were normalized to the current induced by 1 µM ACh. The normalizing concentration of ACh was applied before and after drug application to each oocyte to check for desensitization. Data were rejected if responses to the normalizing dose fell below 75% of the original response. IC₅₀ values were determined using data from four to six oocytes.

Statistical Analysis

Data were analyzed statistically by an analysis of variance followed by the Fisher protected least-significant difference multiple comparison test. For time course studies, Dunnett’s test was used. The null hypothesis was rejected at the 0.05 level. IC₅₀ (antagonist concentration 50%) and ED₅₀ (effective dose 50%) values with 95% confidence limits (CL) were calculated by unweighted least-squares linear regression as described by Tallarida and Murray (1987). Tests for parallelism were calculated according to the method of Tallarida and Murray (1987). If confidence limit values did not overlap, then the shift in the dose-response curve was considered significant.

Results

Antinociception Studies

Tail-Flick Test. Nicotine time course of action after an equipotent dose in male (2 mg/kg) and female (3.5 mg/kg) mice was evaluated in the tail-flick after s.c. injection. As seen in Fig. 1A, no significant difference was seen between male and female mice. Nicotine’s effect disappeared completely within 30 min after s.c. administration in both sexes. Dose-response relationships were then established for nicotine in male and female mice by measuring antinociception at the time of maximal effect (5 min) (Fig. 1B). Nicotine was 3 times less potent in females compared with males. Nicotine
produced a dose-responsive increase in the tail-flick latency in both male and female mice with ED$_{50}$ ($\pm$ CL) values of 1.0 (0.6–1.3) and 2.9 (1.4–5.8) mg/kg, respectively. The basal latencies between male and female mice were not statistically different (2.5 $\pm$ 0.2 and 2.7 $\pm$ 0.2 s for male and female, respectively). Pretreatment with the centrally active non-competitive nicotinic receptor antagonist mecamylamine (1 mg/kg s.c.) 10 min before nicotine blocked its antinociceptive activity in both male and female mice (Fig. 1C). Although mecamylamine seems to be more potent in blocking nicotine’s effects in female mice (AD$_{50}$ ($\pm$ CL) = 0.09 (0.01–0.4) compared with male AD$_{50}$ ($\pm$ CL) = 0.2 (0.04–1.1)), the difference was not significant because confidence limits of the two curves overlapped. A similar sex difference was also observed with epibatidine, a very potent nicotinic agonist. Epibatidine produced a dose-responsive increase in the tail-flick latency (Fig. 2) in both male and female mice with ED$_{50}$ ($\pm$ CL) values of 0.8 (0.5–1.1) and 1.7 (1.2–2.6) mg/kg, respectively. The basal latencies between male and female mice were not statistically different (2.5 $\pm$ 0.2 s for male and 2.7 $\pm$ 0.2 s for female, respectively).

**Hot-Plate Test.** Nicotine was then evaluated in both male and female mice in another acute pain test, the hot-plate assay. Dose-response relationships were established for nicotine in male and female mice by measuring antinociception at the time of maximal effect (5 min) (Fig. 3). Similar to that observed with the tail-flick test, female mice were less sensitive to the effect of nicotine compared with males. The difference of potency between the two sexes was less (1.8 times) than the one determined for the tail-flick test. Nicotine produced a dose-responsive increase in the hot-plate latency in both male and female mice with ED$_{50}$ ($\pm$ CL) values of 0.5 (0.4–0.6) and 0.9 (0.8–1.2) mg/kg, respectively. The basal latencies between male and female mice were not statistically different (12.9 $\pm$ 0.8 and 11.5 $\pm$ 3.2 s for male and female, respectively).

**Antinociceptive Effects after i.t. Injection in the Tail-Flick Test.** Nicotine potency was then evaluated in the tail-flick after i.t. injection in male and female mice. Similar to that observed with s.c. administration, female mice were less sensitive to the effect of nicotine compared with males (Fig. 4A). Nicotine produced a dose-responsive increase in the tail-flick latency in both male and female mice with ED$_{50}$ ($\pm$ CL) values of 11.4 (9.5–13.7) and 23 (15.1–34.2) mg/animal, respectively. To determine whether the sex differences extended to cholinesterase inhibitors, neostigmine was evaluated by i.t. injection. The ED$_{50}$ values for neostigmine-induced antinociception in male and female mice were 0.004 and 0.003 mg/animal, respectively. Therefore, the effects of elevating endogenous ACh were not influenced by gender.

**Other Behavioral Effects of Nicotine**

To further characterize sex differences, additional experiments were conducted to determine nicotine potency in other behavioral responses.

**Effect on Plus-Maze Activity.** Nicotine increased the time spent in the open arm of the maze when administered s.c. to male and female mice (Fig. 5A). Nicotine potency was higher in male mice compared with female mice with ED$_{50}$ ($\pm$ CL) values of 0.40 (0.2–0.6) and 0.95 (0.8–1.2) mg/kg, respectively. No gender differences were seen for total crossings between the different arms in the plus-maze test (data not shown).

**Effect on Body Temperature, Seizure Activity, and Locomotor Activity.** Unlike the antinociceptive and anxiolytic effects, nicotine potency in male and female mice was similar in inducing hypothermia, hypomotility, and seizures production after s.c. administration (Fig. 5, B–D; Table 1). No sex differences were observed in baseline values in the spontaneous activity and body temperature (data not shown).

**In Vivo Interaction of Sex Hormones with Nicotine with Expressed Neuronal Nicotinic Receptors**

The interaction between nicotine and different sex hormones (testosterone, 17$\beta$-estradiol, and progesterone) was studied in the tail-flick test after s.c. administration of nicotine. Time course studies were performed to determine the

![Fig. 2. Antinociceptive effects of epibatidine in male (□) and female (●) mice using the tail-flick test after s.c. administration. Epibatidine dose-response curves were determined in both sexes 10 min after injection. Each point represents the mean ± S.E. of 12 to 16 mice.](Image 310x500 to 559x730)

![Fig. 3. Antinociceptive effects of nicotine in male (□) and female (●) mice using the hot-plate test after s.c. administration. Nicotine dose-response curves in both sexes were determined 5 min after injection. Each point represents the mean ± S.E. of 12 to 16 mice.](Image 45x89 to 296x302)
time of maximal blockade and then dose-response curves for each hormone in blocking nicotine-induced antinociception were carried out to determine the blockade potency.

**Effect of Progesterone.** Progesterone was evaluated for its ability to antagonize a 3.5-mg/kg dose of nicotine in female mice using the tail-flick procedure after i.p. injection. As shown in Fig. 6A, progesterone time dependently blocked nicotine-induced antinociception. The duration of action of progesterone in the tail-flick test was time-dependent with maximum blockade occurring between 3 and 4 h after injection of a dose of 20 mg/kg. Nicotine's effect started to recover within 6 h after pretreatment with progesterone. Additionally, as shown in Fig. 6B, progesterone dose dependently blocked nicotine-induced antinociception with an AD50 (±CL) of 4.8 (2.8–8.4) mg/kg when given s.c. 4 h before nicotine.

Experiments were then carried out to determine whether repeated exposure to progesterone could enhance its blockade potency against nicotine. Female mice were injected with either vehicle or progesterone (10 mg/kg) twice a day for 4 days. Twelve hours after the last injection, the potency of progesterone in blocking a challenge of nicotine was assessed. As shown in Fig. 7, progesterone potency in blocking nicotine-induced antinociception was enhanced as shown by a 3-fold shift to the left of its dose-response curve. The AD50 values (and 95% CL) for vehicle-treated and progesterone-treated animals were 2.4 (1.6–7.5) and 0.8 (0.2–1.4) mg/kg, respectively. Chronic administration of vehicle did increase the potency of progesterone as a nicotinic blocker [4.8 (2.8–8.4) versus 2.4 (1.6–7.5) mg/kg]. This difference was not significant because confidence limits of the two curves overlapped.

**Effect of 17β-Estradiol and Testosterone.** Similar to progesterone, 17β-estradiol time and dose dependently blocked nicotine-induced antinociception in female mice. The duration of effect of 17β-estradiol (20 mg/kg i.p.) was briefer than that of progesterone, with a maximum effect at 3 h after injection and a full recovery of nicotine's effect 6 h later (Fig. 8A). Additionally, as shown in Fig. 8B, 17β-estradiol dose dependently blocked nicotine-induced antinociception with an AD50 (±CL) of 5.5 (4.0–6.6) µg/kg when given s.c. 4 h before nicotine. In contrast to progesterone and 17β-estradiol, testosterone (up to 500 mg/kg i.p.) did not significantly decrease nicotine-induced antinociception at the times (Fig. 9A) and doses tested (Fig. 9B).
Interaction of Sex Hormones with Neuronal Nicotinic Receptors Expressed in Oocytes

Because progesterone and 17\(\beta\)-estradiol blocked nicotine's behavioral effects, their potency as a blocker at expressed neuronal nicotinic receptors was investigated. Progesterone and 17\(\beta\)-estradiol at 50 \(\mu\)M elicited little current when applied for 10 s to oocytes expressing the \(\alpha_4\beta_2\) subunit combinations (2.5 nA). Although they did not activate these expressed receptors, they antagonized the effects of nicotine in a concentration-related manner. The concentration that blocked 50% of the nicotinic current was determined to be 1.8 (1.1–2.9) and 2.0 (1.5–4.3) \(\mu\)M for progesterone and 17\(\beta\)-estradiol, respectively (Fig. 10, A and B).

TABLE 1
Summary of the potency of nicotine in male and female mice in different behavioral tests

<table>
<thead>
<tr>
<th>Pharmacological Effect or Test</th>
<th>Male ED(_{50}) (mg/kg)</th>
<th>Female ED(_{50}) (mg/kg)</th>
<th>Potency Ratio (F/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail-flick after s.c.</td>
<td>1.0 (0.6–1.3)</td>
<td>2.9 (1.4–5.8)</td>
<td>2.9</td>
</tr>
<tr>
<td>Tail-flick after i.t.</td>
<td>11.4 (9.5–13.7)(^b)</td>
<td>23 (15.1–34.2)(^b)</td>
<td>2.0</td>
</tr>
<tr>
<td>Hot-plate</td>
<td>0.50 (0.4–0.6)</td>
<td>0.9 (0.8–1.2)</td>
<td>1.8</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>1.1 (0.5–2.2)</td>
<td>0.85 (0.6–1.9)</td>
<td>0.80</td>
</tr>
<tr>
<td>Hypomotility</td>
<td>0.30 (0.05–0.8)</td>
<td>0.35 (0.15–0.8)</td>
<td>1.2</td>
</tr>
<tr>
<td>Seizures</td>
<td>5.2 (4.8–5.7)</td>
<td>5.8 (4.4–7.4)</td>
<td>1.1</td>
</tr>
<tr>
<td>Plus-maze</td>
<td>0.4 (0.2–0.6)</td>
<td>0.95 (0.8–1.2)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* Potency ratio = female ED\(_{50}\) value/male ED\(_{50}\) value.

\(^b\) ED\(_{50}\) values (±CL) after i.t. administration are expressed as micrograms per animal.

**Discussion**

The primary findings of this study are that there are sex differences in nicotine's acute pharmacological effects, but
these differences are response-dependent. This study also confirms that sex hormones are functional blockers of nico-

tinic receptors in in vivo and in vitro tests. Previous work suggested that gender was an important factor that influ-

ences certain behavioral effects of nicotine, such as those on feeding behavior (Grunberg et al., 1984), locomotor sensitivi-

zation (Booze et al., 1999), and cognition (Kanyt et al., 1999). Among the responses to nicotine where significant sex differ-

ences were observed in our studies are the antinociceptive and the anxiolytic effects of nicotine. Female mice were found less sensitive to the acute effects of nicotine in these tests. Although gender differences in the plus-maze test have been

not described before, the difference in nicotine-induced an-

tinociception agrees well with a recent human study where women were less sensitive to nicotine than men (Jamner et al., 1998).

This sex difference is not limited to nicotine, but other nicotinic agonists such as epibatidine showed a similar differ-

ence. In addition, the fact that a similar sex difference was seen after spinal injection would also confirm the involve-

ment of spinal mechanisms in mediating such a difference. However, no central administration of nicotine was per-

formed in the present study. Furthermore, the lack of sex difference in the case of neostigmine suggests that sex inter-

acted significantly with nicotinic and not muscarinic drugs because neostigmine-induced antinociception is mediated

through muscarinic and not nicotinic receptors (Yaksh et al., 1985; Naguib and Yaksh, 1994).

Proposed Mechanisms Underlying Sex Differences in Nicotine Analgesia. One obvious possibility is that nic-

totine bioavailability might differ between the sexes. Al-

Fig. 8. Blockade of nicotine-induced antinociception in the tail-flick test by 17β-estradiol after i.p. injection in female mice. A, time course of 17β-estradiol's effect on nicotine-induced antinociception (3 mg/kg s.c.) after i.p. administration of 20 μg/kg in mice. B, dose-response blockade of 17β-estradiol after i.p. administration in mice. 17β-Estradiol at different doses was administered i.p. 3 h before nicotine (3.0 mg/kg s.c.) and mice were tested 5 min later. Each point represents the mean ± S.E. of 8 to 12 mice. *p < 0.05 compared with correspondent zero time point.

Fig. 9. Lack of testosterone's effects on nicotine-induced antinociception in the tail-flick test after i.p. injection in male mice. A, effect of testosterone (250 mg/kg i.p.) at different times after injection on nicotine-induced antinociception (3 mg/kg s.c.) in mice. B, effect of testosterone at different doses on nicotine-induced antinociception (3 mg/kg s.c.) 4 h before nicotine. Mice were tested 5 min later. Each point represents the mean ± S.E. of 8 to 12 mice.
though a sex difference in nicotine kinetics is probably not a major influence in humans, there is evidence of sex difference in nicotine metabolism in rats, where studies showed that male rats eliminate nicotine faster than females (Kyerematen et al., 1988; Nwoso and Crooks, 1988). Although plasma and brain concentrations of nicotine were not measured in our studies, our data argue against an exclusive role for pharmacokinetics in accounting for the differential analgesic response of male and female mice to nicotine. Indeed, the following arguments suggest that kinetic factors are probably playing a minor role: 1) the fact that sex differences were not seen in a uniform manner in all behavior responses measured; 2) sex differences were seen with more than one nicotinic agonist (epibatidine and nicotine); and 3) sex differences were seen in nicotine potency in the tail-flick test after i.t. administration.

Another possibility put forward to explain sex differences in nicotine’s effects relates to the influence of pharmacodynamic factors. Recent results suggest that females require higher doses than males to produce nicotine receptor up-regulation (Collins et al., 1988; Koylu et al., 1997; Musachio et al., 1997). Sex differences in receptor up-regulation after chronic exposure to nicotine was reported with only male rats showing up-regulation of brain \(^{3}H\)cytisine binding sites (Koylu et al., 1997). In this study female rats showed a slightly higher baseline density of nicotinic receptors than males.

Several recent reports (Craft and Milholland, 1998; Chiari et al., 1999; Lavand’homme and Eisenach, 1999) that describe sex differences in nicotine-induced antinociception do not agree with the present study. These reports have shown that male rats are less sensitive to nicotine than females in acute and chronic pain models. It is possible that species differences in nicotine metabolism, receptor affinity, density, or functional regulation could explain such contrast between these results and our data. The results with mecamylamine, where no significant difference in its potency in blocking nicotine in both male and female mice was observed, may indicate that regulation of nicotinic receptors involved in nicotine-induced antinociception does not play a major role in the gender difference observed.

Another factor that could underlie sex differences is the influence of gonadal hormones. Our data clearly indicate that sex hormones can modulate the effects of nicotine and nicotinic receptors in a differential manner. There have been a few reports on the influence of hormones on nicotine’s pharmacological effects, and our results agree with their findings. Ke and Lukas (1996) reported that progesterone and estradiol blocked \(^{86}\text{Rb}^+\) efflux in cells expressing muscle and ganglionic nicotinic receptors, and that testosterone failed to inhibit nicotine’s effects even at high concentration. Additionally, progesterone and its A-ring reduced metabolites were reported to be noncompetitive inhibitors on thalamic \(^{86}\text{Rb}^+\) efflux assay that likely measures the functional activity of \(\alpha_4\beta_2\)-type nicotinic receptors (Bullock et al., 1997). A previous report (Valera et al., 1992) showed that progesterone and testosterone were noncompetitive nicotinic blockers in oocyte expressing chick \(\alpha_4\beta_2\) nicotinic receptors, with an IC\(_{50}\) of 9 and 46 \(\text{M}\), respectively. Our results extended these observations and we found that progesterone and \(17\beta\)-estra-diol are functional blockers of nicotinic receptors in vivo and in vitro conditions, with progesterone being a more potent antagonist of \(\alpha_4\beta_2\) receptors (1.8 versus 25 \(\text{M}\)). Testosterone failed to block nicotine’s behavioral effects at very high doses, which correlates well with previous studies. We also report that chronic exposure to progesterone enhances its in vivo effects on nicotine consistent with what is reported in vitro tests (Ke and Lukas, 1996). The increase in progesterone potency after repeated injections could be also due to drug accumulation. But how important might these effects of hormones be on nicotinic receptor function? Our data with progesterone indicate that nicotinic receptor function could be affected by this steroid. Progesterone levels in rat plasma can reach levels between 2.5 and 20 \(\text{M}\) at different stages (Ichikawa et al., 1974; Concas et al., 1999). Under some conditions such as stress and pregnancy, blood levels of progesterone in humans can reach 1 \(\text{M}\) (Hart et al., 1985). In addition, local concentrations of these hormones at receptor sites could be higher because of their lipid-soluble nature (Concas et al., 1999).

In summary, evidence is accumulating that sex differences in nicotine’s effects are important factors to consider in our effort to develop better prevention and treatment strategies in smoking cessation. Indeed, a number of studies have shown that women find it more difficult than do men to quit smoking cigarettes (for review, see Perkins et al., 1999). Our results with nicotine analgesia are important in that regard for several reasons. A number of studies have pointed to nicotine’s analgesic effects as a potential reinforcer of smok-
ing behavior (Fertig and Pomerleau, 1986; Perkins et al., 1994; Jannier et al., 1998) because of the ability of nicotine to reduce pain and discomfort. In addition, Jannier et al. (1998) reported a significant gender difference with men showing a decrease in electrical pain threshold and tolerance after application of nicotine patches but they had no effect in women. Furthermore tolerance was reported to develop to nicotine's antinociceptive effects in animals. Our findings contribute to our search for receptor mechanisms in drug dependence and in the discovery of better pharmacological agents for nicotine dependence.

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References


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