Antinociceptive and Pharmacological Effects of Metanicotine, a Selective Nicotinic Agonist

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ABSTRACT

Metanicotine \([N\text{-methyl-4-(3-pyridinyl)-3-butene-1-amine}]\), a novel neuronal nicotinic agonist, was found to bind with high affinity \((K_i = 24 \text{ nM})\) to rat brain \([3H]\)nicotine binding sites and it generalized to nicotine in a dose-dependent manner in the drug discrimination procedure. Metanicotine produced significant antinociceptive effects in mice and rats subjected to either acute thermal (tail-flick), mechanical (paw-pressure), chemical (para-phenylquinone), persistent (Formalin), and chronic (arthritic) pain stimuli. Metanicotine was about 5-fold less potent than nicotine in the tail-flick test after s.c administration, but slightly more potent after central administration. Its duration of action was longer than that of nicotine. Nicotinic antagonists, mecamylamine and dihydro-\(\beta\)-erythroidine, blocked metanicotine-induced antinociception in the different pain models. However, the antinociceptive effect was not affected by pretreatment with either naloxone or atropine, confirming that metanicotine exerts its antinociceptive effect via nicotinic rather than either opioid or muscarinic mechanisms. In contrast to nicotine, antinociceptive effects of metanicotine were observed at doses that had virtually no effect on spontaneous activity and body temperature in mice. These data indicate that metanicotine is a centrally acting neuronal nicotinic agonist with preferential antinociceptive effects in animals. Thus, metanicotine and related nicotinic agonists may have great potential for development as a new class of analgesics.

The management and treatment of pain is probably one of the most common and yet most difficult aspects of medical practice. Analgesic therapy is currently dominated by the two major classes of analgesic drugs; namely, opioids and nonsteroidal anti-inflammatory drugs. Many improved synthetic variants, as well as improved techniques of administration, have been developed, but there is considerable opportunity for conceptual innovation. Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbances, renal damage (with nonsteroidal anti-inflammatory drugs), respiratory depression, and possibly dependence (with opioids). It is obvious that new molecules designed as analgesic agents are needed.

Activation of cholinergic pathways by nicotine elicit antinociceptive effects in a variety of species (Mattila et al., 1968; Phan et al., 1973; Aceto et al., 1986). Although the effects of nicotine may not extend to all types of pain and appear to depend on the mode of administration, recent observations suggest that cigarette smoking and nicotine reduce pain in humans (Rau et al., 1993; Perkins et al., 1994; Lane et al., 1995; Jammer et al., 1998), implicating a true analgesic component. However, the data in human clinical literature on the relationship between nicotine and pain are inconclusive at best. Most evidence implicates central pathways in the action of nicotine. Indeed, peripherally administered quaternary derivatives of nicotine, which do not readily penetrate the central nervous system (CNS), do not induce antinociception (Aceto et al., 1983). In addition, antagonism of the effect of nicotine is achieved by the centrally and peripherally active antagonist, mecamylamine, but not by the quaternary antagonist, hexamethonium, which crosses the blood-brain barrier poorly (Sahley and Berntson, 1979; Molinero and Del Rio, 1987). Interest in the potential analgesic activity of compounds acting at acetylcholine nicotinic receptors \((\text{nAChRs})\) has recently been stimulated by the discovery of epibatidine, a potent antinociceptive agent isolated from frog skin. Epibatidine is greater than 100-fold more potent than either nicotine or morphine in rodent models of antinociception (Badio and Daly, 1994; Damaj et al., 1994a,b). Unfortunately, nicotine, epibatidine, and most classical nicotinic agonists show poor nAChRs selectivity, which results in a broad spectrum of CNS effects as well as unwanted peripheral (gastrointestinal and cardiovascular) effects. Therefore, nicotinic agonists with selectivity for CNS nAChR might have the beneficial antinociceptive effects of...
nicotine with fewer side effects associated with peripheral nicotinic receptors.

Recent studies with the novel nicotinic agonist metanicotine (known also as RJR-2403; N-methyl-4-(3-pyridinyl)-3-butene-1-amine), suggest that receptor selectivity is a feasible approach (Bencherif et al., 1996; Lippiello et al., 1996). This compound binds with high affinity to $[^{3}H]$nicotine binding sites in rat brain ($K_i = 26 \text{nM}$) but possesses weak affinity (36 $\mu$M) for the $^{125}$I-labeled $\alpha$-bungarotoxin-sensitive nACHR subtype. In vitro functional assays indicate that metanicotine does not stimulate ion flux in rat autonomic ganglia (PC 12 cells) or human muscle (TE671 cells). On the other hand, metanicotine is equal to or better than nicotine as a cognitive enhancer in rats. Metanicotine was 10- to 30-fold less potent than nicotine in eliciting changes in blood pressure, heart rate, and temperature in rats (Lippiello et al., 1996).

In the present study, we investigated the antinociceptive activity and behavioral effects of trans-metanicotine, a novel nicotinic ligand with selectivity for neuronal nAChRs. The antinociceptive effect of metanicotine was investigated using models of acute thermal (mouse tail-flick and hot-plate tests), mechanical (paw-pressure test in rats), and visceral [para-phenylquinone (PPQ)] pain tests. Effects of metanicotine on persistent and chronic pain were assessed using the mouse Formalin test and arthritic pain model, respectively. The effects of metanicotine were compared with those of nicotine in different behavioral models (locomotor activity, drug discrimination, and body temperature measurement), and its sensitivity to different nicotinic antagonists was also assessed. These behavioral models, coupled with receptor binding, offer sufficient opportunity for ascertaining nicotinic effects and evaluating metanicotine as a potential analgesic drug with fewer side effects.

Materials and Methods

Animals

Male ICR mice (20–25 g) and male Sprague-Dawley rats (175–225 g) obtained from Harlan Laboratories (Indianapolis, IN) were used throughout the study. Animals were housed in an American Association for the Accreditation of Laboratory Animal Care-approved facility in groups of six and had free access to food and water. The study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Drugs

Mecamylamine hydrochloride and dihydro-$\beta$-erythroidine hydrobromide were supplied as a gift from Merck Research Laboratories (West Point, PA). (−)-Nicotine was obtained from Aldrich Chemical Company (Milwaukee, WI) and converted to the ditartrate salt, as described by Aceto et al. (1979). Metanicotine oxalate was synthesized as described by Acheson et al. (1980). Atropine sulfate and hexamethonium hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). Naloxone was supplied by the National Institute on Drug Abuse (Washington, DC). All compounds were dissolved in physiological saline (0.9% sodium chloride) and given in a total volume of 1 ml/100 g b.wt. for s.c. injections. All doses are expressed as the free base of the drug. For induction of arthritis in rats, heat-sacrificed Mycobacterium butyricum (0.5 mg/0.5 ml) was purchased from Difco (Detroit, MI).

Intrathecal (i.t.) 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 $\mu$l. The accurate placement of the needle was evidenced by a quick “flick” of the mouse’s tail. In protocols where two sequential injections were required in an animal, the flicking motion of the tail could be elicited with the subsequent injection.

Intraventricular Injections

Intraventricular injections were performed according to the method of Pedigo et al. (1975). Mice were lightly anesthetized with ether and an incision was made in the scalp so that the bregma was exposed. Injections were performed using a 26-gauge needle with a sleeve of PE 20 tubing to control the depth of the injection. An injection volume of 5 $\mu$l was administered at a site 2 mm rostral and 2 mm caudal to the bregma at a depth of 2 mm.

Antinoceptive Tests

Tail-Flick Test. Antinoception 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 established 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 percent maximum possible effect (%MPE, where %MPE = [(test-control)/(20 − control)] × 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 nicotinic ligands for the dose-response evaluation. Antagonism studies were carried out by pretreating the mice with either saline or nicotinic antagonists 5 min before nicotinic agonists. The animals were tested 5 min after administration of the agonist.

Hot-Plate Test. The method is a modification of those 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) maintained at 56.5°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 percentage of maximum possible effect (% MPE, where %MPE = [(test − control)/(20 − control)] × 100). The reaction time was scored when the animal jumped or licked its paws. Eight mice per dose were injected s.c. with metanicotine and tested at various times thereafter to establish a time course.

PPQ Test. The mice were injected s.c. with compounds and 5 min later received an i.p. injection of 2 mg/kg of PPQ solution as described by Pearl et al. (1968). At 10 min after the PPQ injection, the total number of stretches per group was counted within a 1-min period. A stretch is characterized by an elongation of the mouse’s body, development of tension in the muscles of the abdominal region, and an extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of PPQ stretching response. Appropriate vehicles were included.

Formalin Test. The Formalin test was carried out in an open Plexiglas cage, with a mirror placed under the floor to allow an unobstructed view of the paws. Mice were allowed to acclimate for 15 min in the test cage before Formalin injection. Nicotinic analogs or control solution was injected s.c. at different times before the Formalin injection. Each animal was injected with 20 $\mu$l of 2.5% Formalin in the intraplantar region of the right hindpaw. Mice were then observed (two at a time) 0 to 5 min (phase 1) and 20 to 45 min (phase 2) post-Formalin, and the amount of time spent licking the injected paw was recorded. A vehicle-control group was included for each compound or set of compounds.

Arthritic Rats. An arthritic condition closely resembling rheumatoid arthritis in humans can be developed in rats. Either vehicle (paraffin oil/Arlacel A, 85:15) or Freund’s complete adjuvant (heat-sacrificed Mycobacterium butyricum, 0.5 mg) was injected intra-
mally into the plantar aspect of the rat paw and into the base of the tail. The animals remained in their cages for 18 days and were tested on day 19. Inflammation that begins within 24 h proceeds into a generalized polyarthritis within 19 days (Colpaert et al., 1982; Milian et al., 1988). Paw-pressure baseline measurements on day 19 indicated that arthritic rats were more sensitive to mechanical nociception than nonarthritic rats. The paw-pressure test consisted of gently holding the body of the rat while the hindpaw was exposed to increasing mechanical pressure. The Analgesy-Meter (Ugo-Basile; Varese, Italy) is designed to exert a force on the paw that increases at a constant rate, in a manner similar to the Randall-Selitto (1957) test of mechanical nociception. The force was applied to the hindpaw that was placed on a small plinth under a cone-shaped pusher with a rounded tip. The operator depressed a pedal switch to start the mechanism that exerted force. The force in grams at which the rat struggled was defined as the paw-pressure threshold. The baseline paw pressure was measured before injecting vehicle or drug. After measurement of baseline paw-pressure thresholds, the animals were tested at different times, after the s.c. administration of vehicle or nicotinic agonists. Antinociception was expressed as the paw pressure (in grams), with each repeated measure’s time point representing the mean response of eight rats. The upper limit of 500 g was imposed for the experiments.

Behavioral Testing

Locomotor Activity. Mice were placed into individual Omnitech photocell activity cages (28 × 16.5 cm) 5 min after s.c. administration of either 0.9% saline, nicotine, or metanicotine. Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 10 min. Data were 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 different times after the s.c. injection of either metanicotine or nicotine for time-course determinations. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21 to 24°C from day to day.

Nicotine Drug Discrimination in Rats. Rats were individually housed in a temperature-controlled environment and were maintained on a diet (Agway Rodent Chow) that restricted their body weight to approximately 85% of their free feeding weight. Water was available ad libitum in the home cages. A two-lever operand drug-discrimination paradigm (VI 15) was carried out in eight operand chambers (4 Lafayette model 80001 and 4 BRS/LVE model sec 002). Reinforcement was a Bioserv 45-mg precision dustless pellet. Data were collected automatically by two Commodore 64 microcomputers.

Rats were trained to respond on one lever after an s.c. injection of (−)nicotine (0.4 mg/kg) and another lever after an s.c. injection of saline. Rats were placed in an operand chamber 5 min after injection. The specific procedure for training rats to discriminate between nicotine and saline has been described previously (Rosecrans, 1989). Animals were required to meet a criterion of 3 successive days of 80% or greater correct-lever responding before testing was initiated. Injections were given 5 min before placing the animal in the operand chamber. The schedule of injections was determined using a Latin Square design. Dose-response curves were determined for nicotine 5 min after s.c. injections.

[^3H](−)Nicotine Binding In Vitro

[^3H](−)Nicotine binding assays in rat brain were performed in vitro, according to the method of Scimeca and Martin (1988) with minor modifications. Tissue homogenate was prepared from whole rat brain (minus cerebellum) in 10 vol of ice-cold 0.05 M Na-K phosphate buffer (pH 7.4) and centrifuged (12,100g, 4°C) for 30 min. The pellet was then resuspended in 20 vol of ice-cold glass-distilled water and allowed to remain on ice for 60 min before being centrifuged as before. The resulting pellet was then resuspended to a final tissue concentration of 10 mg/ml of buffer. Membranes from whole brain (0.2 ml of final suspension) were incubated at 4°C for 2 h with phosphate buffer and[^3H]nicotine at the indicated concentrations in a total volume of 1 ml. Nonspecific binding was determined in the presence of 100 μM unlabeled nicotine. The incubation was terminated by rapid filtration through a Whatman GF/C glass fiber filter (preoashed overnight in 0.1% poly-L-lysine to reduce radioligand binding to the filters). Filters were washed twice with 3 ml of the buffer, and radioactivity on the filters was measured using a liquid scintillation spectrometer. The B_max and K_D values, obtained from Scatchard analysis, were determined via the KELL package of binding analysis programs for the Macintosh computer (Biosoft, Milltown, NJ). The ability of nicotinic agonists to displace 1.5 nM[^3H]nicotine binding was determined in the presence of increasing concentrations of metanicotine and nicotine.

Statistical Analysis

Data were analyzed statistically by ANOVA followed by the Fisher pair least significant difference multiple-comparison test. The null hypothesis was rejected at the .05 level. For the time-course studies, each animal was used once. Data were analyzed by a two-factor ANOVA. ED_{50} values with 95% CLs for behavioral data were calculated by unweighted least-squares linear regression as described by Tallarida and Murray (1987).

Results

Binding Experiments

The Scatchard analysis of[^3H]nicotine binding provided a K_D of 1.3 ± 0.15 nM and B_max of 245 ± 46 fmol/mg protein. Both nicotine and metanicotine inhibited binding of[^3H]nicotine to rat brain membranes. The K_I values were, respectively, 1.4 ± 0.20 and 24 ± 12 nM.

Antinociception Studies

Tail-Flick Test. The onset of action for metanicotine (10 mg/kg, s.c.) in the tail-flick test was rapid, with maximum antinociception occurring between 10 and 15 min. The duration of metanicotine-induced antinociception was longer, compared with that of nicotine. Indeed, nicotine’s effect disappeared completely within 30 min after s.c. administration in mice (Fig. 1A). However, as illustrated in Fig. 1A, metanicotine’s effect gradually diminished to 20% ± 11 at 60 min (significantly different from the 5-min time point and from saline) and disappeared completely within 120 min after injection. Dose-response relationships were established for nicotine and metanicotine in mice by measuring antinociception at the time of maximal effect (Fig. 1B). Nicotine and metanicotine produced a dose-responsive increase in the tail-flick latency (Fig. 1B) with an ED_{50} (CL) of 1.5 (0.9–1.8) and 7.2 (3.6–14.0) mg/kg, respectively. Metanicotine was five times less potent than nicotine as an antinociceptive agent in mice after s.c. administration in the tail-flick test. However, this difference in potency was reversed after spinal or supraspinal administration of the drugs. Indeed, 5 min after i.t. injection, nicotine and metanicotine elicited a dose-responsive antinociceptive effect in the tail-flick test (Fig. 2A). The ED_{50} values (CL) were determined to be 12 (10.5–17.0) and 4 (2.6–5.6) μg/mouse for nicotine and metanicotine, respectively (Table 1). Moreover, metanicotine was also more potent than nicotine in eliciting a dose-dependent antinociception after intracerebroventricular (i.c.v.) administration,
with an ED$_{50}$ value (CL) of 9.0 (6.7–11.8) mg/mouse, whereas an ED$_{50}$ value of 14.0 (9–20) mg/mouse was determined for nicotine.

Pretreatment with the centrally active noncompetitive nicotinic receptor antagonist mecamylamine (1 mg/kg, s.c.) 10 min before metanicotine (15 mg/kg) blocked its antinociceptive activity. Similarly, the competitive nicotinic blocker dihydro-β-erythroidine (2 mg/kg, s.c.) blocked metanicotine-induced antinociception. However, pretreatment with naloxone (1 mg/kg, s.c.), an opioid antagonist, and atropine (10 mg/kg, s.c.), a muscarinic antagonist, failed to significantly block metanicotine’s effect (Table 2). By themselves, these various antagonists did not produce any significant effect on the tail-flick latencies at the doses and times used.

**Hot-Plate and PPQ Tests.** Dose-response relationships were established for nicotine and metanicotine in mice by measuring antinociception at the time of maximal effect (5 min after injection; Fig. 3A) in the hot-plate test. Five minutes after nicotine administration (s.c.), a dose-dependent antinociceptive effect was observed and the ED$_{50}$ value (CL) was determined to be 0.75 (0.6–0.9) mg/kg. Metanicotine was less active than nicotine, with only 50% antinociception at a dose of 40 mg/kg. When mice were pretreated with nicotine and metanicotine, abdominal stretching behaviors, as measured in the PPQ test, were inhibited in a dose-related manner (Fig. 3B) yielding ED$_{50}$ value of 0.2 (0.1–0.37) and 1.6 (0.55–4.4) mg/kg. Furthermore, mecamylamine at the dose of 1 mg/kg significantly blocked the effects of nicotine and metanicotine in the PPQ test (data not shown).

**Formalin Test.** The effect of systemic (s.c.) nicotine and metanicotine treatment on both phase 1 (0–5 min) and phase 2 (20–45 min) of the Formalin test was investigated 5 min after injection. In both phase 1 (Fig. 4A) and phase 2 (Fig. 4B), metanicotine dose dependently attenuated nocifensive responding, as indicated by an overall significant effect of treatment [early phase: $F_{2,40} = 14.47$, $p < .0001$; late phase: $F_{2,39} = 8.89$, $p < .0001$]. However, nicotine was more potent than metanicotine in both phases, as determined by ED$_{50}$ values of 0.25 (0.18–0.28) and 5.4 (5.0–5.9) mg/kg for phase 1, and 0.75 (0.35–1.05) and 3 (2.6–3.6) mg/kg for phase 2, respectively. The antinociceptive effect of metanicotine (18 mg/kg, s.c.) in both phases 1 and 2 was blocked by pretreatment with mecamylamine (1 mg/kg, s.c.; Table 3).

**Arthritic Pain Model in Rats.** Freund’s complete adjuvant caused a significant reduction in paw-pressor threshold (Table 4). These results indicate that the arthritic rats were significantly more sensitive to mechanical nociception than nonarthritic rats. In addition, the reduction in paw-pressure threshold was reversed in rats 24 h after treatment with...
indomethacin at a dose of 10 mg/kg, given orally. In nonarthritic rats (Fig. 5A), metanicotine and nicotine elicited antinociception in a dose-related manner with an ED50 value (CL) of 0.03 (0.02–0.04) and 1.9 (1.2–3.1) mg/kg, respectively. In arthritic rats, metanicotine also elicited a significant antinociceptive effect (Fig. 5B) to mechanical nociception that lasted for more than 60 min (Fig. 6). However, nicotine was more potent than metanicotine in arthritic rats, as determined by ED50 values of 0.05 (0.04–0.07) and 3.7 (2.3–5.9) mg/kg, respectively. The results indicate that metanicotine and nicotine elicited a similar degree of antinociception in nonarthritic and arthritic rats. The antinociceptive effects of metanicotine and nicotine in both arthritic and nonarthritic rats were blocked by pretreatment with mecamylamine (data not shown).

Behavioral Studies

Effect on Body Temperature and Locomotor Activity. Metanicotine decreased body temperature (Fig. 7A) in a dose-related manner, and the ED50 values (Table 1) at the time of maximal effect (15 min after injection) showed that it was 68 times less potent than nicotine in inducing hypothermia in mice. Furthermore, pretreatment with mecamylamine, at 1 mg/kg s.c., did not significantly decrease metanicotine-induced hypothermia (metanicotine at 40 mg/kg = −2.9 ± 0.2°C and

### Table 1
Summary of the potency of metanicotine compared with (−)-nicotine in behavioral models and receptor binding

<table>
<thead>
<tr>
<th>Pharmacological Effect or Test</th>
<th>(−)-Nicotine</th>
<th>Metanicotine</th>
<th>Potency Ratio M/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail-flick after s.c.</td>
<td>1.5 (0.9–1.5)</td>
<td>7.2 (3.6–14.0)</td>
<td>4.8</td>
</tr>
<tr>
<td>Tail-flick after i.t.</td>
<td>12.0 (10.5–17.0)</td>
<td>4.0 (2.6–5.6)</td>
<td>0.3</td>
</tr>
<tr>
<td>Tail-flick after i.c.v.</td>
<td>14.0 (9–20)</td>
<td>9.0 (6.7–11.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Hot-plate</td>
<td>0.50 (0.43–0.49)</td>
<td>50% @ 40</td>
<td>&gt;80</td>
</tr>
<tr>
<td>PPQ test</td>
<td>0.2 (0.1–0.37)</td>
<td>1.6 (0.55–4.4)</td>
<td>8</td>
</tr>
<tr>
<td>Formalin test—phase 1</td>
<td>0.25 (0.18–0.28)</td>
<td>5.4 (5.0–5.9)</td>
<td>21.6</td>
</tr>
<tr>
<td>Formalin test—phase 2</td>
<td>0.75 (0.35–1.05)</td>
<td>3.2 (2.6–3.6)</td>
<td>4</td>
</tr>
<tr>
<td>Paw pressure—nonarthritic rats</td>
<td>0.03 (0.02–0.04)</td>
<td>1.9 (1.2–3.1)</td>
<td>63.3</td>
</tr>
<tr>
<td>Paw pressure—arthritic rats</td>
<td>0.05 (0.04–0.07)</td>
<td>3.7 (2.3–5.9)</td>
<td>74</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>1.2 (0.5–2.2)</td>
<td>82 (75.5–97.0)</td>
<td>68.3</td>
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<tr>
<td>Hypomotility</td>
<td>0.60 (0.5–0.72)</td>
<td>2% @ 42</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Drug discrimination</td>
<td>0.3 (0.1–0.6)</td>
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Receptor affinity

|                       | 1.4 ± 0.20 | 24 ± 12 | 17.1  |

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### Table 2
Effect of various antagonists on metanicotine-induced antinociception in mice, using in the tail-flick test after s.c. administration

Results are expressed as %MPE and represent the mean ± S.E. of six to eight mice.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>%MPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Metanicotine</td>
<td>77 ± 14*</td>
</tr>
<tr>
<td>Mecamylamine (1 mg/kg)</td>
<td>Metanicotine</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine (2 mg/kg)</td>
<td>Metanicotine</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>Atropine (10 mg/kg)</td>
<td>Metanicotine</td>
<td>70 ± 12*</td>
</tr>
<tr>
<td>Naloxone (1 mg/kg)</td>
<td>Metanicotine</td>
<td>67 ± 14*</td>
</tr>
</tbody>
</table>

* Dose of metanicotine was 15 mg/kg.

* p < .05 from vehicle/vehicle.

Fig. 3. Antinociceptive effects of metanicotine (●) and nicotine (○) measured 5 min after s.c. administration in other acute pain models. A, dose-response curves in the hot-plate test. Each point represents the mean ± S.E. of 8 to 12 mice. B, dose-response curves in the PPQ test. Shown are the percentage of inhibition of PPQ stretching responses of 8 to 12 mice/group.
mecamylamine. In addition, nicotine at the time of maximal effect (5 min after injection) dose dependently decreased locomotor activity in mice after s.c. treatment (see Table 1). However, contrary to nicotine, metanicotine showed little effect on locomotor activity (4% decrease at 42 mg/kg) after s.c. administration (Fig. 7B).

**Discrimination Studies in Rats.** In rats trained to discriminate between vehicle and nicotine (0.4 mg/kg), nicotine increased the percentage of responses on the drug-appropriate bar in a dose-related manner with an ED$_{50}$ of 0.15 mg/kg (0.2–0.58). Metanicotine given s.c. elicited nicotine-like responses in these rats, as illustrated in Fig. 8A. However, the dose-response curve for metanicotine indicated that it was 12 times less potent than nicotine. Indeed, at the time of maximal effect (5 min after drug s.c. administration), the ED$_{50}$ value (CL) for metanicotine was determined to be 1.9 (0.65–5.6) mg/kg. A decrease in nicotine-like responding was seen at a higher dose of metanicotine (12.5 mg/kg). At the dose of 5 mg/kg of metanicotine, the response rate was decreased by 20% (Fig. 8B). The discriminative effect of metanicotine in rats was significantly blocked by mecamylamine (1 mg/kg, s.c.). Animals treated with saline before metanicotine (4 mg/kg) elicited 81.6% appropriate drug-lever responding, whereas those treated with mecamylamine before metanicotine produced 9.5.6% drug-lever responding. Mecamylamine by itself did not produce any significant generalization to the nicotine cue at the dose and time used.

**Discussion**

Recent receptor studies suggested a clear selectivity of the nicotinic agonist metanicotine for central rather than peripheral nicotinic receptors (Bencherif et al., 1996). This observation is consistent with the present results, which indicate...
that this compound exhibits antinociceptive action at doses that are devoid of pheripheral effects.

Our data demonstrate that metanicotine elicited antinociceptive effects in mice and rats subjected to either acute thermal (tail-flick), mechanical (paw-pressure), chemical (PPQ), persistent (Formalin), and chronic (arthritis) pain stimuli. Metanicotine was about 5-fold less potent than nicotine in the tail-flick test after s.c administration, but slightly more potent (2-3-fold times) after central administration (i.t. and i.c.v.). Such potency differences observed after peripheral and central administration suggest the possible involvement of either metabolic or biodispositional factors in metanicotine’s effects. Furthermore, the duration of metanicotine-induced antinociception was almost four times longer than nicotine. Metanicotine was 8-fold less potent than nicotine in the PPQ and Formalin tests, which compare favorably to the tail-flick test. This difference between the tail-flick and hot-plate tests suggest that spinal sites play an important role in metanicotine’s antinociceptive effects (Irwin et al., 1951).

In contrast to nicotine, antinociceptive effects of metanicotine were observed at doses that had virtually no effect on spontaneous activity and body temperature in mice (Table 2). A factor of 70-fold was observed in the two responses between the two drugs. Similar dissociation was also reported for metanicotine between its memory-enhancement and peripheral effects (Lippiello et al., 1996).

Further characterization of the pharmacological effects of metanicotine using different approaches revealed that neuronal nicotinic receptors are involved in its actions. Indeed, metanicotine was found to bind with high affinity ($K_i = 24$ nM) to rat brain [$^3$H]nicotine binding sites, and it generalized to nicotine in a dose-dependent manner in the drug discrimination procedure. Mecamylamine and dihydroy-β-erythroidine blockade of metanicotine-induced antinociception in the different pain models provide further support for metanicotine’s anti-inflammatory component of nicotine’s action is more pronounced that that of metanicotine. Surprisingly, metanicotine failed to produce maximal antinociceptive effects in the hot-plate test. This difference between the tail-flick and hot-plate tests suggest that spinal sites play an important role in metanicotine’s antinociceptive effects (Irwin et al., 1951).
Data suggest that nicotine in the tail-flick test (Marubio et al., 1999). These observations do not directly address the identity of specific nicotinic receptor subtypes that mediate metanicotine’s antinociceptive effects, comparison of the in vitro and in vivo results suggests some possibilities. For example, metanicotine binds with high affinity to rat brain \(^{3}H\)nicotine binding sites which contain \(\alpha_2\) and \(\beta_2\) subunits. In addition, it was reported that metanicotine has a low affinity to \(\alpha_2\) receptors (\(K_I \sim 36 \mu M\); Bencherif et al., 1996). Finally, a good selectivity between the potency of metanicotine in the different behavioral measures was found. Taking into consideration the in vitro profile and the separation between the different in vivo pharmacological effects, it is reasonable to speculate that activation of \(\alpha_2\beta_2\) receptor subtype is at least partially involved in metanicotine-induced antinociception. However, the involvement of other receptor subtypes such as \(\alpha_7\)- and \(\alpha_4\)-containing receptors is also possible.

In conclusion, the selective nicotinic agonist metanicotine has antinociceptive effects in mouse and rat models of acute and chronic pain. Failure of metanicotine to produce some of nicotine’s motor and hypothermic effects suggests that it may have an improved safety profile relative to nicotine. This unique pharmacological profile suggests that it is possible to separate antinociceptive and undesirable effects of nicotinic receptor activation. Moreover, metanicotine serves as a valuable probe for establishing the role of receptor subtypes in nicotine’s actions.

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References


Fig. 8. Generalization tests with of metanicotine (○) and nicotine (■) in rats trained to discriminate 0.4 mg/kg of nicotine from saline. A, mean (± S.E.) percentage of nicotine-correct responding of rats after s.c. injection of different doses of nicotinic agonists. B, responses per second for dose-response curve in nicotine-trained rats. Rats were tested 5 min after injection of nicotine and metanicotine. Each point represents the mean ± S.E. of 8 to 10 rats. *p < .05, compared with correspondent vehicle point.

The receptor subtypes at which nicotinic agonists exert their antinociceptive actions have been recently investigated. Knockout mice deficient in the \(\alpha_4\) and \(\beta_2\) nicotinic acetylcholine receptor subunits were found to be insensitive to nicotine on the hot-plate test and display diminished sensitivity to nicotine in the tail-flick test (Marubio et al., 1999). These data suggest that \(\alpha_4\beta_2\) subunits of the nAChR are involved in nicotine-induced antinociception. Although the present studies do not directly address the identity of specific nicotinic receptor subtypes that mediate metanicotine’s antinociceptive effects, comparison of the in vitro and in vivo results suggests some possibilities.


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