Effect of Dextrometorphan and Dextrorphan on Nicotine and Neuronal Nicotinic Receptors: In Vitro and in Vivo Selectivity

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ABSTRACT

The effects of dextrometorphan and its metabolite dextrorphan on nicotine-induced antinociception in two acute thermal pain assays after systematic administration were evaluated in mice and compared with that of mecamylamine. Dextrometorphan and dextrorphan were found to block nicotine's antinociception in the tail-flick and hot-plate tests with different potencies (dextrometorphan is 10 times more potent than its metabolite). This blockade was not due to antagonism of N-methyl-D-aspartate receptors and/or interaction with opiate receptors, since selective drugs of these receptors failed to block nicotine's analgesic effects. Our results with the tail-flick and hot-plate tests showed an interesting in vivo functional selectivity for dextrometorphan over dextrorphan. In oocytes expressing various neuronal acetylcholine nicotinic receptors (nAChR), dextrometorphan and dextrorphan blocked nicotine activation of expressed $\alpha_3\beta_4$, $\alpha_4\beta_2$, and α_7 subtypes with a small degree of selectivity. However, the in vivo antagonistic potency of dextrometorphan and dextrorphan in the pain tests does not correlate well with their in vitro blockade potency at expressed nAChR subtypes. Furthermore, the apparent in vivo selectivity of dextrometorphan over dextrorphan is not related to its in vitro potency and does suggest the involvement of other mechanisms. In that respect, dextrometorphan seems to behave as another mecamylamine, a noncompetitive nicotinic receptor antagonist with a preferential activity to $\alpha_3\beta_4^*$ neuronal nAChR subtypes.

Dextrometorphan is structurally related to the morphinan opioid levorphanol and is widely known as a centrally acting non-narcotic antitussive agent. Although dextromethorphan produces little analgesia by itself, it was found to potentiate the antinociceptive effects of opiates and reduce tolerance and physical dependence to morphine (Mao et al., 1996). In addition, Glick et al. (2001) have shown that both dextrometorphan and its major metabolite, dextrorphan, decreased nicotine, methamphetamine, and morphine self-administration in rats after s.c. administration. These multiple pharmacological and behavioral effects may be related to the ability of dextrometorphan and dextrorphan to interact with various receptor systems. Dextrometorphan has little or no opioid activity but binds with high affinity to σ sites (Klein and Musacchio, 1989). It binds, along with dextrorphan, with low affinity to the phencyclidine site of the NMDA receptor with dextrorphan having a 10-fold higher affinity at the site (Franklin and Murray, 1992). In addition, dextrometorphan and dextrorphan are both antagonists at NMDA glutamate receptors (Murray and Leid, 1984). They were also recently shown to block $\alpha_3\beta_4$ neuronal nicotinic receptors in a noncompetitive fashion (Hernandez et al., 2000) with dextrometorphan 3-fold less potent than dextrorphan. These reports suggest that dextrometorphan and/or dextrorphan may hold promise for understanding nicotine pharmacology and nicotine dependence. However, further studies are needed to investigate the modulation of nicotine's pharmacological effects by dextrometorphan and its metabolite and to compare their effects on various neuronal nAChRs. Although a wide range of pharmacological effects are elicited after nicotine administration, we were particularly interested in nicotine's antinociceptive action.

Activation of cholinergic pathways by nicotine elicits antinociceptive effects in a variety of species (Phan et al., 1973; Aceto et al., 1986). There is strong evidence that the antinociceptive effect of nicotine can occur via activation of nAChRs expressed in the central nervous system and peripheral sensory neurons (Aceto et al., 1986; Iwamoto, 1989; Christensen and Smith, 1990, 1991; Iwamoto and Marion, 1993; Flores et al., 1996; Khan et al., 1997; Bitner et al., 1998; Damaj et al., 1998). The antinociceptive effect of nicotine is of short duration (5–45 min) and occurs at relatively high doses (0.5–2 mg/kg). In addition, tolerance develops after repeated administration as measured in acute thermal pain models (Damaj and Martin, 1996). It is

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; nAChR, acetylcholine nicotinic receptor; %MPE, percent maximum possible effect; CL, confidence limits; MK-801, dizocilpine.

still unclear, however, which nicotinic receptor populations are responsible for the antinociceptive effects of nicotine. In large part, this ambiguity results from a lack of available subtype-specific agonists and antagonists. Nonetheless, multiple experimental approaches such as transgenic mice and antisense studies have proved insightful. These mechanistic studies conducted to date have largely focused on the role of $\alpha_4\beta_2$* receptors in tests of acute, thermal nociception. The reported results suggest that $\alpha_4\beta_2$ receptor subtypes are components of the nicotinic response in the hot-plate test and to a lesser extent in the tail-flick assay (Marubio et al., 1999; Bitner et al., 2000). The role of non-$\alpha_4\beta_2$ nAChRs in nicotinic analgesia is still, however, largely unknown. Recent studies have reported that the α_7 antagonists failed to block the antinociceptive effects of various nicotinic agonists in the tail-flick test after central administration in rats and mice indicating little involvement of α_7 subtypes (Rao et al., 1996; Damaj et al., 1998).

In the present study, we investigated the extent to which dextrometorphan and its metabolite could alter the antinociceptive activity of nicotine in two acute thermal pain assays, the tail-flick and the hot-plate tests. The potential antagonism of nicotine-induced antinociception by dextrometorphan and dextrorphan was assessed after systemic (s.c. and i.p. injections) administration. The route of administration of dextrometorphan can have a substantial impact on its metabolism. Much higher levels of dextrorphan are generated after an i.p. injection of dextrometorphan compared with the levels after s.c. injection (Wu et al., 1995). The comparison of different routes of administration will be helpful in understanding the relative extent to which dextrometorphan and dextrorphan mediate the effects of dextrometorphan on nicotine's antinociceptive activity. The effects of dextrometorphan and its metabolite were compared with that of mecamylamine, a noncompetitive nicotinic antagonist with a preferential activity at $\alpha_3\beta_4$ receptor subtypes (Papke et al., 2001). In addition, the selectivity of their nicotinic response was examined by testing the effects of various dextrometorphan structural analogs, opiate and/or NMDA receptor modulators, on nicotine-induced antinociception in mice.

In vivo studies were complemented with the in vitro profiling of dextrometorphan and dextrorphan on various neuronal nicotinic receptor subtypes. The antagonistic activity of dextrometorphan and dextrorphan was assessed at the $\alpha_4\beta_2$, $\alpha_3\beta_4$, and α_7 nicotinic receptor subtypes expressed in oocytes.

Materials and Methods

Animals

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

Drugs

Mecamylamine hydrochloride was supplied as a gift from Merck Research Labs (West Point, PA). (−)-Nicotine was obtained from Aldrich Chemical Co. (Milwaukee, WI) and converted to the ditartrate salt as described by Aceto et al. (1979). Dizocilpine (MK-801), dextrometorphan, and dextrorphan were purchased from Sigma/RBI (Natick, MA). Acetylcholine and other chemicals used in the oocyte buffer solutions were obtained from Sigma-Aldrich (St. Louis, MO). Naloxone, (+)-morphine, codeine isomers, and levorphanol were sup-

plied by the National Institute on Drug Abuse (Washington, DC). All drugs were dissolved in physiological saline (0.9% sodium chloride). All doses are expressed as the free base of the drug.

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 percent maximum possible effect (%MPE), where %MPE = [(test − control)/(10 − control)] × 100.

Hot-Plate Test. Mice were placed into a 10-cm wide glass cylinder on a hot plate (Thermojust Apparatus) maintained at 55.0°C. Two control latencies at least 10 min apart were determined for each mouse. The normal latency (reaction time) was 8 to 12 s. Antinociceptive response was calculated as %MPE, where %MPE = [(test − control)/(40 − control) × 100]. The reaction time was scored when the animal jumped or licked its paws. To minimize tissue damage, a maximum latency of 40 s was imposed.

Groups of 8 to 12 animals were used for each dose and for each treatment. Antagonism studies were carried out by pretreating the mice with either saline, dextrometorphan, or dextrorphan 15 min before nicotine. This 15-min time was determined in separate experiments to be the optimum pretreatment time for dextrometorphan and dextrorphan (data not shown). The animals were tested 5 min after administration of nicotine. Opiate agonists and antagonists as well as NMDA antagonists were evaluated for their ability to antagonize nicotine in the tail-flick test. Codeine isomers, levorphanol (a structural analog of dextrometorphan with opiate activity), and (+)-morphine were tested at the highest inactive doses in the tail-flick test. Naloxone (a nonselective opiate antagonist) was given at 1 mg/kg, a dose that is largely reported to block morphine's analgesic effects.

Oocyte Expression Studies

Molecular Biology. The rat cDNAs for the α_3, β_4, α_4, and β_2 nAChRs were in pcDNAI/neo Polymerase T7, sk-Polymerase T3, psp64 Polymerase SP6, and psp65 Polymerase SP6 vectors, respectively. The human α_7 nAChR clone was in a pMXT expression vector. The vectors were linearized and used as templates for run-off transcription from the SP6 promoter using standard techniques.

Oocyte Extraction and Injection. *Xenopus laevis* oocytes were extracted from anesthetized females and placed in ND-96 medium (96 mM NaCl, 2 mM KCl, 1 mM MgCl$_2$, 1.8 mM CaCl$_2$ H$_2$O, 5 mM HEPES, 2.5 mM sodium pyruvate, 0.5 mM theophylline, and 10 mg/liter gentamicin, adjusted to pH 7.5). The oocyte clusters were incubated in 0.2% collagenase (type IA, Sigma-Aldrich) in ND-96 medium for defolliculation. Oocytes were agitated at 18.5°C for 4 h and afterward rinsed with Barth's medium (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO$_3$, 15 mM HEPES, pH 7.6). The oocytes were left to recover for 24 h in L-15 oocyte medium before injection of cRNA. L-15 oocyte media were obtained from Specialty Media (Phillipsburg, NJ). Approximately 10 ng of cRNA was injected into individual oocytes in volumes of ~100 nl using a manual injector (Nanoject; Drummond Scientific, Broomall, PA). For $\alpha_4\beta_2$ and $\alpha_3\beta_4$ nicotinic receptors, cRNA for the two subunits was injected, mixed in a 1:1 ratio. The oocytes were incubated at 17°C for 3 to 7 days in ND-96 medium prior to electrophysiological recording.

Electrophysiology. Oocyte preparation and recording was performed as previously described by Coates and Flood (2001). Current recordings were made from whole oocytes at room temperature (19–23°C) using a GeneClamp 500 two-microelectrode voltage-clamp amplifier with an active ground circuit (Axon Instruments, Inc., Union City, CA). The recording electrodes were pulled from glass capillaries (Drummond Scientific) to obtain a resistance between 1 and 5 MΩ when filled with 3 M KCl. The Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM BaCl$_2$, 10 mM HEPES, 1 μM atropine, pH 7.4) used

for recordings contained atropine to prevent muscarinic receptor stimulation and barium in place of calcium to avoid current amplification by calcium activated chloride currents. Oocytes were clamped at a holding potential of -60 mV unless otherwise indicated and held in a 125-μl cylindrical channel. All drugs were applied by perfusion at a flow rate of 4 ml/min. The oocyte was pre-exposed to dextromethorphan or dextrorphan for 2 min unless otherwise noted and tested with a 2-s agonist application in the continued presence of dextromethorphan. Activation was complete during the application period. To minimize the contribution of nAChR desensitization, 3 min passed between acetylcholine applications to α_7 nAChRs, and 5 min passed between agonist applications to $\alpha_4\beta_2$ and $\alpha_3\beta_4$ nAChRs. Using this time interval, steady-state recordings could be obtained in control experiments. A baseline control response to the agonist was measured before and after each agonist-antagonist coapplication. The response to dextromethorphan was compared with the average of the preceding and following agonist response.

Statistical Analysis of the Curves. Inhibitory concentration-response relationships for the inhibition were constructed by calculating the current recorded in the presence of antagonist as a percentage of that elicited by the agonist alone. The resulting data were fitted to a modified Hill equation, $y = y_{max}/(1 + (x/IC_{50})^n)$, where IC_{50} is the concentration of antagonist that elicited 50% of the maximal inhibition, y_{max} is the maximal current elicited by the agonist, and n is the Hill coefficient. The data points obtained at each concentration were averaged, and the calculated mean and standard error were fit to a modified Hill equation. Clampex 7 (Axon Instruments, Inc.) was used for data acquisition, and Microcal Origin 5.0 (OriginLab Corp, Northampton, MA) was used for graphics and statistical calculation. $p < 0.05$ was considered significant, and data were represented as mean $\pm$ S.E.M.

Statistical Analysis. Statistical analysis of all analgesic studies was performed using either t test or analysis of variance with Tukey's post hoc test when appropriate. All differences were considered significant at $p < 0.05$. AD_{50} values with 95% CL for behavioral data were calculated by unweighted least-squares linear regression as described by Tallarida and Murray (1987).

Results

Effects of Dextrometorphan and Dextrorphan on Nicotine-Induced Antinociception in the Tail-Flick Test: Time Course, Potency, and Selectivity. Dextrometorphan and dextrorphan were evaluated for their ability to antagonize a 2.5 mg/kg dose (an ED_{84} dose) of nicotine in the tail-flick procedure after i.p. injection. We first carried out a time course study of dextrometorphan and dextrorphan antagonism with doses of 10 and 50 mg/kg i.p., respectively. The duration of antagonism by dextrometorphan and dextrorphan in the tail-flick test was time-dependent with maximum blockade occurring between 5 and 30 min for the 10 and 50 mg/kg dose, respectively. The two drugs antagonized nicotine for up to 2 h. Indeed, as illustrated in Fig. 1, nicotine's effect recovered within 60 min after pretreatment with dextrometorphan, but was still significantly different from control 120 min after the dose of 50 mg/kg of dextrorphan. Based on the time course results, subsequent studies were carried out by pretreating the mice with dextrometorphan or dextrorphan 15 min before nicotine. We then determined their potency of blocking nicotine's effects at this maximum pretreatment time.

As shown in Fig. 2, both dextrometorphan and dextrorphan dose-dependently blocked nicotine-induced antinociception when given i.p. 15 min before nicotine with determined AD_{50} values of 2.4 (1.4–4.1) and 25 (22–29) mg/kg, respectively (Table 1). By themselves, dextrometorphan and dex-
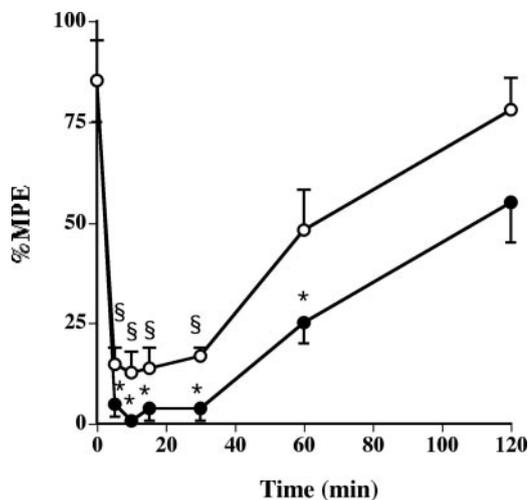

Fig. 1. Time course of (○) dextrometorphan and (●) dextrorphan effect on nicotine-induced antinociception (2.5 mg/kg) after i.p. administration of 10 and 50 mg/kg in mice, respectively. Each point represents the mean $\pm$ S.E.M. of 8 to 12 mice. $*$ and §, $p < 0.05$ compared with the corresponding zero time point.
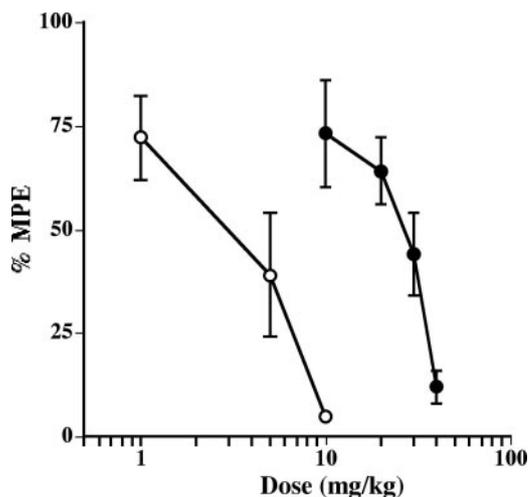

Fig. 2. Dose-response blockade of nicotine-induced antinociception in the tail-flick test by (○) dextrometorphan and (●) dextrorphan after i.p. administration in mice. Drugs at different doses were administered i.p. 15 min before nicotine (2.5 mg/kg s.c.), and mice were tested 5 min later. Each point represents the mean $\pm$ S.E.M. of 8 to 12 mice.

trorphan did not significantly change tail-flick basal latencies at the indicated doses and times.

The potency of dextrometorphan in blocking nicotine-induced antinociception was then determined after s.c. injection. As presented in Fig. 3, dextrometorphan blocked nicotine's effects in a dose-related manner with an AD_{50} value of 0.8 (0.6–0.9) mg/kg. This value indicates that dextrometor-

TABLE 1

Summary of the blockade potency of dextrometorphan, dextrorphan, and mecamylamine on nicotine-induced antinociception in the tail-flick and hot-plate tests after systemic administration in mice

AD_{50} values ($\pm$CL) were calculated from the dose-response and expressed as milligrams per kilogram. Each dose group included six to eight animals.

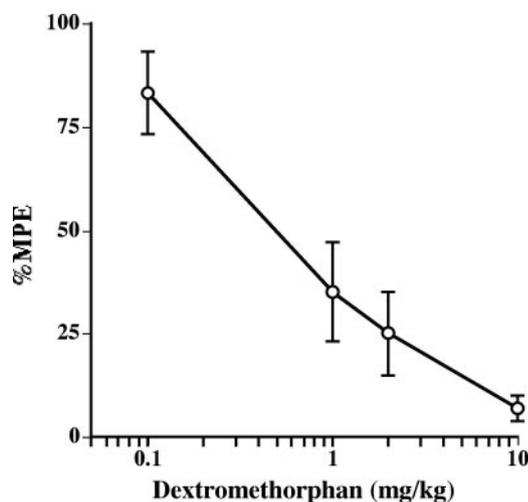| Antagonist	Tail-Flick Test (AD_{50} mg/kg $\pm$ CL)	Hot-Plate Test (AD_{50} mg/kg $\pm$ CL)
Dextrometorphan	2.4 (1.4–4.1)	13 (6.8–21)
Dextrorphan	25 (22–29)	24 (18–32)
Mecamylamine	0.09 (0.05–0.13)	0.8 (0.5–1.1)

Fig. 3. Dose-response blockade of nicotine-induced antinociception in the tail-flick test by dextrometorphan after s.c. administration in mice. Dextrometorphan at different doses was administered s.c. 15 min before nicotine (2.5 mg/kg s.c.), and mice were tested 5 min later. Each point represents the mean ± S.E.M. of 8 to 12 mice.

phan given s.c. is 3 times more potent in blocking the antinociceptive effect of nicotine compared with that determined after i.p. injection [0.8 (0.6–0.9) versus 2.4 mg/kg] (Table 1).

To ascertain that the dextrometorphan/nicotine in vivo interaction was specific to nicotinic systems, opiate analogs and NMDA antagonists were evaluated for their ability to antagonize nicotine in the tail-flick test. As shown in Table 2, MK-801, naloxone, codeine isomers, levorphanol (a structural analog of dextrometorphan with opiate activity), and (+)-morphine given at behaviorally active doses failed to significantly block nicotine-induced antinociception in mice. Mecamylamine, given s.c. at 1 mg/kg in the same experimental conditions, totally blocked nicotine's effects in the tail-flick test.

Effects of Dextrometorphan and Dextrorphan on Nicotine-Induced Antinociception in the Hot-Plate Test. In contrast to the tail-flick test, the difference in potency of dextrometorphan and dextrorphan in blocking nicotine-induced antinociception as measured by the hot-plate test was not significantly different. When given i.p. 15 min before nicotine, dextrometorphan blocked nicotine's effects in the hot-plate test in a dose-dependent manner with an AD_{50} of 13 (6.8–22) mg/kg. Dextrorphan blocked nicotine's effects

TABLE 2
Effect of MK-801, an NMDA antagonist, and various opiate analogs on nicotine-induced antinociception in the tail-flick test after s.c. administration

Vehicle or the pretreatment drugs were administered s.c. 10 to 15 min before nicotine (2.5 mg/kg s.c.), and the mice were tested 5 min later after the second injection. Each point represents the mean ± S.E.M. of 6 to 12 mice.

Treatment	%MPE
Vehicle/vehicle	7 ± 2
Vehicle/nicotine	84 ± 10
Dizocilpine (2 mg/kg)/nicotine	81 ± 10
Naloxone (1 mg/kg)/nicotine	78 ± 17
Levorphanol (0.5 mg/kg)/nicotine	75 ± 16
(+)-Codeine (50 mg/kg)/nicotine	88 ± 7
(−)-Codeine (50 mg/kg)/nicotine	87 ± 5
(+)-Morphine (10 mg/kg)/nicotine	72 ± 15
Mecamylamine (1 mg/kg)/nicotine	5 ± 1

in a dose-dependent manner with an AD_{50} of 24 (18–32) (Fig. 4).

Effects of Mecamylamine on Nicotine-Induced Antinociception in the Tail-Flick and Hot-Plate Tests. The potency of mecamylamine, a noncompetitive nicotinic antagonist, in blocking nicotine's effects in the two acute thermal pain assays was compared with that of dextrometorphan and dextrorphan after s.c. treatment. Nicotine-induced antinociception after systemic administration in mice (2.5 mg/kg), in the tail-flick and hot-plate tests, was blocked by mecamylamine in a dose-dependent manner (Fig. 5). Calculation of the AD_{50} showed that mecamylamine is 9 times more potent in blocking the antinociceptive effect of nicotine in the tail-flick than the hot-plate test [0.09 (0.05–0.13) versus 0.8 (0.5–1.1) mg/kg] (Table 1).

Interaction of Dextrometorphan and Dextrorphan with Expressed Neuronal Nicotinic Receptors. Since dextrometorphan and dextrorphan differentially blocked nicotine's antinociceptive effects, their potencies as blockers at various neuronal nicotinic receptors were investigated. At a concentration of 100 μM, they did not elicit any current response when applied for 60 s to oocytes expressing the $\alpha_4\beta_2$, α_7, or $\alpha_3\beta_4$ subunit combinations (data not shown). Although they did not activate these expressed receptors, dextrometorphan and dextrorphan antagonized the effects of acetylcholine in a concentration-related manner (Fig. 6). In addition, dextrometorphan displayed differential potency in blocking the various nicotinic receptors with the $\alpha_3\beta_4$ receptor being the most sensitive. Indeed, the concentration of dextrometorphan that blocked 50% of the nicotinic current (IC_{50}) was determined to be 0.7 (0.1) and 3.9 (0.2) μM for $\alpha_3\beta_4$ and $\alpha_4\beta_2$ receptors, respectively (Table 2). α_7 receptors were also sensitive to dextrometorphan blockade with an IC_{50} value of 2.5 (0.2) μM (Fig. 6 and Table 3). The relative selectivity seen with dextrometorphan was less evident with dextrorphan. The IC_{50} values for dextrorphan were determined to be 1.3 (0.1) and 3.0 (0.5) μM for $\alpha_3\beta_4$ and $\alpha_4\beta_2$ receptors, respectively. α_7 receptors were the least sensitive
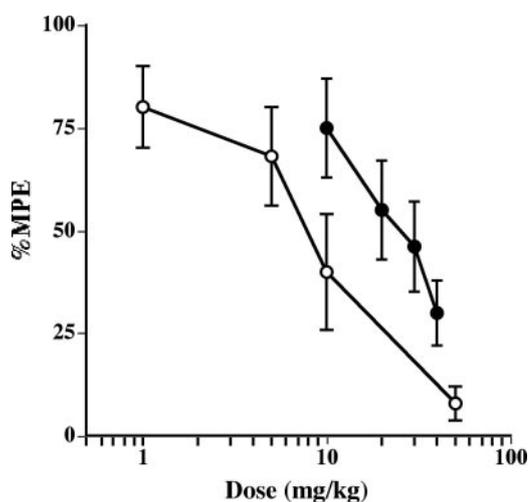

Fig. 4. Dose-response blockade of nicotine-induced antinociception in the hot-plate test by (○) dextrometorphan and (●) dextrorphan after i.p. injection in mice. Dextrometorphan and dextrorphan at different doses were administered i.p. 15 min before nicotine (2.5 mg/kg s.c.), and mice were tested 5 min later. Each point represents the mean ± S.E.M. of 8 to 12 mice.
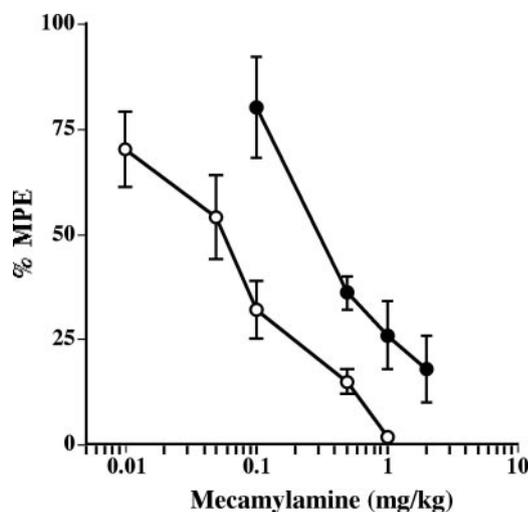
Fig. 5. Dose-response blockade of nicotine-induced antinociception in (○) the tail-flick and (●) hot-plate tests by mecamylamine after s.c. injection in mice. Mecamylamine at different doses was administered s.c. 10 min before nicotine (2.5 mg/kg s.c.), and mice were tested 5 min later. Each point represents the mean ± S.E.M. of 8 to 12 mice.

to dextrorphan blockade with an IC_{50} of 4.3 (0.2) μM (Fig. 6 and Table 3).

Discussion

The primary findings of this study are that dextromethorphan and its principle metabolite dextrorphan are blockers of nicotinic antinociceptive effects and functional inhibitors of various neuronal nAChR subtypes. These results confirm and expand the findings of previous studies that reported dextromethorphan and dextrorphan blocked $\alpha_3\beta_4$-expressed nicotinic receptor subtypes in HEK-293 cells (Hernandez et al., 2000) and decreased nicotine self-administration in rats (Glick et al., 2001). The higher potency in blocking $\alpha_3\beta_4$ subtypes expressed in oocytes found in our study compared with that reported by Hernandez et al. (2000) (IC_{50} of 0.7 μM in oocytes versus 8.9 μM in cells for dextromethorphan) can be explained by use of different agonists to stimulate nAChRs [acetylcholine in our studies compared with nicotine in Hernandez et al. (2000)]. In addition, different post-translational mechanisms between the two expression systems may impact the sensitivity of drugs on nicotinic receptors.

The blockade of nicotine's analgesic effects by dextromethorphan and dextrorphan seems to occur independently of their interaction with NMDA and/or opiate receptors. MK-801, a noncompetitive NMDA antagonist, failed to block nicotine's analgesic effects. Furthermore, the fact that dextromethorphan was more potent than dextrorphan in the tail-flick test and equipotent in blocking the hot-plate effects supports the lack of involvement of NMDA receptors. If NMDA receptor blockade was the major mechanism responsible for dextromethorphan's effects, dextrorphan should have been more potent than dextromethorphan in blocking nicotine's actions in both tests because of its higher affinity to these receptors. Opiate mechanisms also have a minor role in dextromethorphan's effects, since opiate drugs such as (+)-morphine, codeine isomers, and dextromethorphan structural analogs with opiate properties, failed to significantly affect nicotine-induced antinociception. Finally, our results with expressed nAChRs demonstrate clearly that dextrometor-

phan and dextrorphan block nicotine's activation of $\alpha_3\beta_4$, $\alpha_4\beta_2$, and α_7 neuronal nAChRs. Taken together, our results suggest that the pronounced effects that dextromethorphan and dextrorphan have on nicotine's analgesic actions in vivo are most likely related to their antagonism of nAChRs.

The present findings, together with the results of Glick et al. (2001) and Hernandez et al. (2000), showed that dextrometorphan, widely available as an over-the-counter cough suppressant, is a nicotinic antagonist. Our results with two different routes of administration (s.c. and i.p.) as well as the time course of nicotine blockade suggest that dextrorphan, the major metabolite of dextromethorphan which possesses a comparable activity and lower toxicity to the parent compound (Fossati et al., 1995), seems to mediate to a certain extent the effects of dextromethorphan on nicotine's analgesic actions. However, the participation of other dextrometorphan metabolites such as 3-methoxymorphinan and 3-hydroxymorphinan in its antagonistic effects cannot be excluded.

Our results with the tail-flick and hot-plate tests showed an interesting difference in in vivo functional potency for dextromethorphan and dextrorphan. First, they block nicotine's antinociception effects in the tail-flick test with different potencies (dextromethorphan is 10 times more potent that its metabolite). Second, dextromethorphan, but not dextrorphan, was found significantly more potent in blocking nicotine-induced antinociception in the tail-flick than the hot-plate test after systemic administration (a ratio of 5), similar to the nicotinic antagonist, mecamylamine. These observations suggest that dextromethorphan possesses some selectivity over dextrorphan for neuronal nicotinic receptors underlying these two nicotinic analgesic responses. The potency ratio in the tail-flick/hot-plate of dextromethorphan ($r = 5$), dextrorphan ($r = 0.9$), and mecamylamine ($r = 12$) correlates well with their in vitro $\alpha_3\beta_4/\alpha_4\beta_2$ blockade potency ratio of 5.5, 2, and 4 (Papke et al., 2001), respectively. In addition, the potency of dextromethorphan in blocking nicotine-induced antinociception in the tail-flick test after s.c. injection increased 3-fold (Table 1) compared with that obtained after i.p. administration. This difference can be explained by the fact that higher levels of dextrorphan are generated after i.p. injection of dextromethorphan compared with the levels after s.c. injection of dextromethorphan (Wu et al., 1995). These findings suggest that $\alpha_3\beta_4$ nAChR subtypes underlie the action for dextromethorphan in blocking nicotine's effects in the tail-flick test. However, the in vivo antagonistic potency of dextromethorphan, dextrorphan, and mecamylamine in the tail-flick test (see Table 1) does not correlate well with their in vitro blockade potency at $\alpha_3\beta_4$ neuronal nAChR subtypes (Table 3). A similar conclusion can also be reached for $\alpha_4\beta_2$ or α_7 nAChR subtypes in the hot-plate test. The involvement of metabolites into dextromethorphan and dextrorphan in vivo effects may help in explaining this discrepancy. Additionally, differences are likely to exist between the receptors expressed in oocytes and those found in vivo, such as additional complex subunit arrangements, post-translational modifications, etc.

The present in vitro functional findings indicate that the various neuronal nAChRs differ little in their sensitivity to inhibition by dextromethorphan and dextrorphan, which does not suggest a particular selectivity toward any of the subtypes tested. The apparent in vivo selectivity of dextrometor-
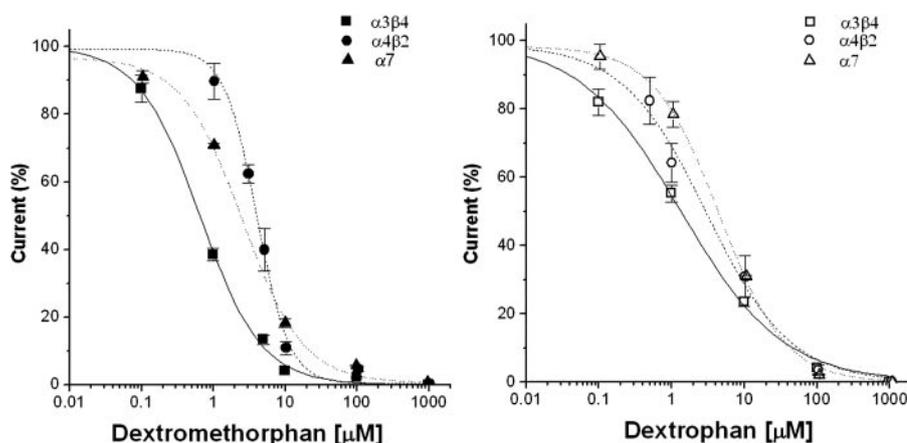
Fig. 6. Concentration dependence of dextrometorphan and dextrorphan inhibition of $\alpha_3\beta_4$, $\alpha_4\beta_2$, and α_7 nAChR subtypes. Responses of different neuronal nicotinic receptors to an EC_{90} concentration of acetylcholine (100 μM for $\alpha_3\beta_4$, $\alpha_4\beta_2$, and 1000 μM for α_7 nAChRs) were recorded in the absence and presence of different concentrations of dextrometorphan and dextrorphan. Oocytes were held at -60 mV.

TABLE 3

Summary of the blockade potency IC_{50} values mean $\pm$ S.E.M. of dextrometorphan and dextrorphan for inhibition of various nAChR functions in oocytes

Nicotinic Receptor	Dextrometorphan		Dextrorphan	
	IC_{50} (Mean $\pm$ S.E.M.)	Hill Coefficient	IC_{50} (Mean $\pm$ S.E.M.)	Hill Coefficient
	μM		μM	
$\alpha_3\beta_4$	0.7 $\pm$ 0.1	1.0 $\pm$ 0.05	1.3 $\pm$ 0.1	0.6 $\pm$ 0.04
$\alpha_4\beta_2$	3.9 $\pm$ 0.2	1.9 $\pm$ 0.19	3.0 $\pm$ 0.5	0.8 $\pm$ 0.09
α_7	2.5 $\pm$ 0.2	1.0 $\pm$ 0.08	4.3 $\pm$ 0.2	1.0 $\pm$ 0.04

phan over dextrorphan is hard to explain with its in vitro potency and suggest the involvement of other mechanisms. In that respect, dextrometorphan seems to behave as another mecamylamine, a noncompetitive nicotinic receptor with a preferential activity to $\alpha_3\beta_4$* neuronal nAChR subtypes.

At this point, it is difficult to predict exactly which nicotinic receptor subtypes are the most important targets for nicotinic analgesia. However, our present results along with previous reports (Marubio et al., 1999; Bitner et al., 2000) have shed some light on this question. Contrary to our results with the hot-plate test where $\alpha_4\beta_2$ subtypes have major involvement, the tail-flick test data provide strong evidence that at least two mechanisms of nicotinic receptor-mediated spinal antinociception involve $\alpha_3\beta_4$ subtype- and $\alpha_4\beta_2$ subtype-containing receptors. In conclusion, the nAChR subtypes involved in the antinociceptive effects of nicotinic agonists vary as a function of the type of pain.

Acknowledgments

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