ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine]: A Novel, Orally Effective Antinociceptive Agent Acting via Neuronal Nicotinic Acetylcholine Receptors: II. In Vivo Characterization

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ABSTRACT

The antinociceptive effects of ABT-594, a novel nicotinic acetylcholine receptor (nAChR) ligand, were examined in rats in models of acute thermal (hot box) and persistent chemical (formalin test) pain. Also, the effects of ABT-594 treatment on motor function and electroencephalogram (EEG) were determined. In the hot box and formalin test (i.e., phase 1 and 2), acute treatment with ABT-594 (0.03, 0.1 and 0.3 \( \mu \)mol/kg i.p.) produced significant dose-dependent antinociceptive effects. In the hot box, the efficacy of ABT-594 was maintained after a repeated dosing paradigm (5 days b.i.d. i.p.). ABT-594 was fully efficacious in the formalin test when administered before formalin, and also retained significant efficacy (0.3 \( \mu \)mol/kg i.p.) when administered after formalin injection. The antinociceptive effects of ABT-594 in the hot box and formalin tests were attenuated by pretreatment with the nAChR antagonist, mecamylamine, and in animals treated with the nAChR antagonist, chlorisondamine, given centrally (10 \( \mu \)g/rat i.c.v. 5 days before), but not in animals pretreated with the opioid receptor antagonist, naltrexone. Acute treatment with ABT-594 produced an initial decrease in open-field locomotor activity, which was absent in animals dosed repeatedly (5 days b.i.d.) with ABT-594. Also, acute treatment with ABT-594 decreased body temperature and decreased the amount of time the animals could maintain balance in an edge-balance test. These effects were no longer present in animals dosed repeatedly with ABT-594. At antinociceptive doses, ABT-594 produced activation of free running EEG in contrast to the sedative-like effects of morphine. Full antinociceptive efficacy was maintained in both the hot box and formalin tests after oral administration, whereas the effects on motoric performance were attenuated. In conclusion, these data demonstrate that ABT-594 is a potent antinociceptive agent with full efficacy in models of acute and persistent pain and that these effects are mediated predominately by an action at central neuronal nAChRs. In addition, antinociceptive effects were maintained after repeated dosing, whereas effects of ABT-594 on motor and temperature measures were attenuated in animals treated repeatedly with ABT-594. Thus, compounds acting at nAChRs may represent a novel approach for the treatment of a variety of pain states.

The use of opioids in pain management is limited by side effects such as constipation and sedation, as well as by dependence, tolerance and scheduling issues (Cherny, 1996). Alternative therapies such as the NSAIDs avoid some of the liabilities of opioids, yet they also have toxicities (e.g., dyspepsia and peptic ulcer) which can limit the clinical usefulness of this class (Singh et al., 1994). An ideal analgesic agent would possess the full analgesic efficacy of an opioid such as morphine, yet be devoid of the side-effect and scheduling liabilities associated with opioid use.

The discovery and characterization of epibatidine has provided the basis for a novel class of analgesic agents acting via nAChRs. Epibatidine is an alkaloid found in the skin of a species of Ecuadorian frogs (Badio et al., 1994; Spande et al., 1992). In models of acute pain such as the hot plate, \((\pm)\)-epibatidine is as efficacious as morphine in producing antinociceptive effects but has 100-fold greater potency (Sullivan and Bannon, 1995). The antinociceptive effects of epibatidine are prevented by pretreatment with the centrally acting nAChR antagonist mecamylamine, but not by the peripher-

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ABBREVIATIONS: NSAIDS, nonsteroidal antiinflammatory drugs; nAChRs, nicotinic acetylcholine receptors; sal, saline; mec, mecamylamine; ntx, naltrexone; chlor, chlorisondamine; nic, nicotine; EEG, electroencephalogram; FFT, Fast Fourier Transform; REM, rapid eye movement; i.c.v., intracerebroventricular.
ally acting nAChR antagonist hexamethonium, which suggests the involvement of central neuronal nAChRs in the antinociceptive effects of epibatidine (Qian et al., 1993). In addition, the antinociceptive effects of epibatidine are not prevented by pretreatment with opioid antagonists such as naloxone, which indicates lack of opioid receptor involvement. Although the potent antinociception of epibatidine has validated the possibility of developing an analgesic agent acting via nAChRs with an efficacy similar to morphine, the clinical development of epibatidine as an analgesic is prohibited because of toxic side effects (e.g., cardiovascular) (Sullivan et al., 1994a, b).

Although the nAChR ligand, (-)-nicotine, previously has been reported to be effective in preclinical models of nociception (Aceto et al., 1983; Caggiula et al., 1995; Davis et al., 1993), (-)-nicotine is at least 100-fold less potent than epibatidine in acute models of pain (Qian et al., 1993) and typically has a short duration of action (Caggiula et al., 1995). Similarly, the therapeutic use of (-)-nicotine for the treatment of pain is unlikely because of its side effects such as the pressor effect on the cardiovascular system (Benowitz, 1992).

Evidence that the beneficial and side-effect liabilities of neuronal nAChR ligands can be separated has a well-founded, neurobiological basis (Arneric, 1998; McGeehee and Role, 1996). The neuronal nAChR is a pentameric structure believed to be composed of two alpha and three beta subunits (Cooper et al., 1991). This is distinct from the nAChR found at the neuromuscular junction that is composed of alpha, beta, gamma, delta and epsilon subunits (Changeux et al., 1992). Although the predominant neuronal nAChR subtype in the central nervous system is alpha-4 beta-2 (Flores et al., 1992), transcripts for other nAChR subunits such as alpha-3, alpha-5, alpha-7 and beta-4 in neuronal tissue (Seguela et al., 1993; Wada et al., 1989, 1990) allow the possibility of numerous neuronal nAChR subtypes in discrete neuronal regions subserving distinct functional roles (Flores et al., 1996). The stable transfection of cell lines with nAChR subtypes indicates that (-)-nicotine as well as (±)-epibatidine are relatively nonselective in activation of proposed neuronal nAChRs, and both ligands are potent agonists at ganglionic nAChRs, which may underlie some of the associated toxicities (Sullivan et al., 1994a). In addition, (±)-epibatidine is also a relatively potent ligand at nAChRs at the neuromuscular junction, which may also contribute to some of its liabilities. The toxic and potentially beneficial (e.g., antinociceptive) effects of nAChR ligands such as epibatidine and (-)-nicotine can be dissociated and may be mediated by interactions of these compounds with distinct nAChR subtypes. Development of selective nAChR compounds may provide agents with improved safety profiles relative to existing nAChR ligands such as (±)-epibatidine.

Development of a nAChR ligand as a potential analgesic would require a nAChR compound with the full antinociceptive activity of (±)-epibatidine and with substantially reduced toxicities. ABT-594 may possess reduced toxicities (Decker et al., in press; Holladay et al., 1998) relative to epibatidine based on enhanced selectivity for neuronal nAChRs compared with the ganglionic-like and neuromuscular nAChR subtypes (Donnelly-Roberts et al., 1998). In the current study we investigated the antinociceptive activity and behavioral effects of ABT-594, a novel nAChR ligand with selectivity for neuronal nAChRs (Bannon et al., 1998; Donnelly-Roberts et al., 1998; Holladay et al., 1998).

Materials and Methods

Subjects

Male Sprague-Dawley rats (Charles River, Portage, MI) weighing 200 to 400 g were used for all experiments. These animals were housed in AAALAC-approved facilities at Abbott Laboratories in a temperature-regulated environment with lights on between 700 and 2000 h. Food and water were available ad libitum at all times except during testing. In all other experiments, rats were housed in groups of 4/cage. All testing was done following procedures outlined in protocols approved by Abbott’s Institutional Animal Care and Use Committee.

Behavioral Procedures

Hot box. For assessing nociceptive responses to an acute thermal stimulus, a commercially available paw thermal stimulator was used (Anesthesiology Research Laboratory, Department of Anesthesiology, University of California at San Diego, La Jolla, CA). This device has been described previously (Dirig and Yaksh, 1995) and is based on the initial work of Hargreaves et al. (1988). Rats were placed in Plexiglas cubicles that were located on a glass surface of the apparatus. The surface of the glass was maintained at 30°C. A thermal stimulus was applied to the bottom of the rear foot of the rat via a movable focused projection bulb. The stimulus current was maintained at 4.8 amps. The latency until the animal moved its foot from the stimulus was recorded automatically by use of photodiode motion sensors. In the current studies, a 20-s cut-off was used to limit possible tissue damage after exposure to the stimulus. For measurements, the following protocol was used. Six animals were used in each run. For any given measure (e.g., time point), one foot of each of the six animals was tested and then the process was repeated for the opposite foot. Mean values for the response were computed based on the two scores.

All studies began with a 20-min acclimation period. After the acclimation period, a baseline measure was determined for each animal. After determination of base line, treatments were administered and measures were taken at various time points after treatment (e.g., 15, 30 and 45 min). For clarity, data were collapsed over time for statistical analysis (unless otherwise noted).

Formalin test. After a 20-min period of acclimation to individual cages, 50 μl of a 5% formalin solution was injected s.c. into the dorsal aspect of one of the rear paws, and the rats were then returned to the clear observation cages suspended above mirror panels. Rats were observed for either a continuous period of 60 min or for periods of time corresponding to phase 1 and phase 2 of the formalin test. Phase 1 of the formalin test was defined as the period of time immediately after injection of formalin until 10 min after the formalin injection (i.e., 0–10 min after formalin). Phase 2 was defined as the 20-min period from 30 to 50 min after formalin injection. The investigator recorded nociceptive behaviors in the injected paw of four animals during the session by observing each animal for one 15-s observation period during each 1-min interval. Nociceptive behaviors recorded included flinching, licking or biting the injected paw. In dose-response studies, ABT-594 (or saline) was administered 5 min before injection of formalin. In antagonist studies, the antagonists or saline were administered 10 min before ABT-594 treatment.

Measurement of locomotor activity. Spontaneous activity was assessed in an open field (41 × 41 cm). Horizontal (locomotion) and vertical activity (rearing) were measured by a photobeam detector system (San Diego Instruments, San Diego, CA) and beam breaks were accumulated for 60 min. These data were combined subsequently into four 15-min blocks for statistical analysis. Rats were placed in the test chambers within 1 to 2 min after compound administration.
Measurement of motor coordination and body temperature. Motor coordination was assessed by determining the rat's ability to maintain balance on the top of a vertical wall (i.e., edge task). This task was developed in our laboratory. An apparatus with four walls joining at 90° angles and open on the top and bottom was used. Each wall was 37 cm high and 40 cm long and constructed of 6-mm-thick Plexiglas. To begin the test, the animal was placed straddling the top surface of one of the walls with one forepaw and one rear paw on each side and held in this position until it was stable. Once the experimenter released the animal, a timer was started and the latency to fall from the wall was recorded. If the animal fell in less than 20 s, it was placed back on the wall for a second trial. In these cases, the better of the two scores was used. Animals that did not fall in 120 s were removed from the apparatus and assigned a score of 120 s. In the repeated dosing experiment, after completion of the edge task, body temperature was recorded with a rectal thermometer (VSI Tele-Thermometer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH.).

Repeated Dosing Studies

Repeated dosing studies were conducted in the hot box and motor coordination paradigms. For these studies, rats were dosed with either saline or ABT-594 (0.3 μmol/kg i.p.) b.i.d. for 5 days. Treatments were separated by approximately 6 h (i.e., morning and afternoon). In the hot box experiment, animals were tested in the morning and afternoon on days 1, 2, and 5. For each test, a base-line measure was recorded, and then animals were tested 15, 30, and 45 min after treatment. For the afternoon treatment on day 5, all animals received a challenge dose of ABT-594 (0.3 μmol/kg i.p.) before being tested. For the motor coordination experiment, animals were tested only in the afternoon on day 5.

EEG Procedures

All surgical procedures for implanting of EEG electrodes were conducted under aseptic conditions. Rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital (Abbott Laboratories, North Chicago, IL). Bilateral drill holes were made in the skull to accommodate epidural electrodes at the following coordinates (in mm from bregma): AP = +2.0, −2.0, −6.0; L all 3.0. Holes for reference and ground electrodes were placed AP = −11.0 and AP = +4.0 near the centerline, respectively. Stainless steel screw electrodes were threaded into the skull and the screws were soldered to the sockets of a miniature connector. Dental acrylic was used to anchor the connector to the skull. Rats were allowed to recover at least 10 days before experiments began.

The rats were recorded inside Plexiglas chambers and allowed free movement within the chambers by use of swivel commutators (Dragonfly Inc., Silver Spring, MD). EEG signals were amplified by AC differential amplifiers (AstroMed-Grass Instruments, Quincy, MA) with low and high bandpass filters set at 1 and 100 Hz, respectively. Signals from the active electrodes were compared with the distant reference electrode. EEG data were recorded by computer-based acquisition software (Brainwave Systems, Broomfield, CO; Stellate Systems, Montreal, Canada) at a sampling rate of 256 Hz. The effects of the test substances were determined by analyzing 1 to 50 Hz EEG amplitude obtained from recordings over the left parietal cortex. The amplitude in microvolts (μV) was calculated by use of Fast Fourier Transform (FFT) equations incorporated in the acquisition software. Compound dosing for the EEG experiments was as follows: ABT-594, 0.03, 0.1, 0.3 μmol/kg; morphine, 42.0 μmol/kg; and caffeine, 50.0 μmol/kg. Doses of ABT-594 were selected on the basis of efficacy in rats on several analgesia tests. All treatments were administered intraperitoneally, and one treatment was administered before each test session. EEG experiments were conducted by use of a within-subjects design; thus, each rat received all the treatments on separate days in a pseudo-random order with at least 48 h between treatments. The EEG was recorded for 50 min immediately after drug treatment.

Chlorisondamine Treatment

Rats were anesthetized with sodium pentobarbital (55 mg/kg i.p.). Animals were placed in a David Kopf student stereotaxic instrument (Tujunga, CA) with the skull on an even horizontal plane. A micro-injection of phosphate-buffered saline or chlorisondamine (10 μg/rat) was made into the left lateral ventricle. The volume for the i.c.v. injection was 5 μl injected for 30 s. Antinociceptive testing in the hot box began 5 days after injection of chlorisondamine. With the same rate, testing in the formalin test began 14 days after injection of chlorisondamine.

Compounds

ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine] and A-98593 [(S)-5-(2-azetidinylmethoxy)-2-chloropyridine] were synthesized at Abbott Laboratories (Abbott Park, IL) as described by Hollanday et al. (1998). Benzoate, tosylate and monohydrochloride salts of ABT-594 were used. Mecamylamine, hexamethonium and naltrexone were obtained from Sigma (St. Louis, MO). All compounds were dissolved in sterile 0.9% saline for systemic treatment and injection volume was 1 ml/kg for i.p. administration. For the p.o. administration, the injection volume was 3 ml/kg. For i.c.v. injections, chlorisondamine (Ciba Geigy, Summit, NJ) was dissolved in sterile phosphate-buffered saline.

Statistics

Data were analyzed by t tests or analysis of variance followed by Fisher’s protected least significant difference test for evaluating pairwise comparisons. A repeated measures one-way analysis of variance with drug dose as the repeated measure was used for analysis of ABT-594 FFT data. A two-tailed paired t test was used for analysis of morphine and caffeine FFT data. Statistical analyses on data were performed with StatView and SuperAnova software (Abacus Concepts, Inc., Berkeley, CA).

Results

Hot box. The effects of ABT-594 and morphine in the hot box were examined after intraperitoneal administration (fig. 1). Data for A-98593, the enantiomer of ABT-594, also are presented. Because A-98593 was tested in a separate experiment (i.e., different control groups) the data are presented as change from control. An overall significant effect of dose was found with ABT-594 and morphine [F(6, 35) = 7.167, P < .0001] as well as A-98593 [F(3, 22) = 8.094, P < .0008] with data collapsed over time (i.e., 45 min). Analysis indicated significant effects with all doses tested of ABT-594 (0.03–0.3 μmol/kg), A-98593 (0.06–0.3 μmol/kg) and morphine (2.4–24 μmol/kg).

![Fig. 1. ABT-594, morphine and A-98593 (enantiomer of ABT-594) produce significant antinociceptive effects in the hot box after systemic (i.p.) administration. Values represent mean change from control ± S.E.M.; n = 8/group. *different from saline, P < .05.](Image)
After oral administration of ABT-594 an overall significant dose effect \( F(3,23) = 16.775, P < .0001 \) was found (fig. 2). Further analysis indicated a significant effect with ABT-594 at 0.19 \( \mu \text{mol/kg} \) i.p. In a separate experiment, the duration of a 0.3 \( \mu \text{mol/kg} \) p.o. dose of ABT-594 was 2 h (data not shown).

In animals treated repeatedly (b.i.d. for 5 days) with either ABT-594 or saline, a challenge dose of ABT-594 (0.3 \( \mu \text{mol/kg} \) i.p.) produced significant antinociceptive effects in the hot box \( F(1,10) = 42.583, P < .0001 \) (table 1). Similar efficacy was observed in both treatment groups as evidenced by lack of a difference between these groups in the effect of ABT-594 treatment \( F(1,10) = 2.371, P = .155 \).

Administration of the nAChR antagonist, mecamylamine (1.5 \( \mu \text{mol/kg} \) i.p.) 15 min before ABT-594 (0.3 \( \mu \text{mol/kg} \) i.p.) blocked the antinociceptive effect (table 2). Pretreatment with the opioid receptor antagonist, naltrexone (2.65 \( \mu \text{mol/kg} \) i.p.), did not block ABT-594-induced antinociception (table 3).

To investigate the involvement of neuronal nAChRs, the effects of ABT-594 (0.1 \( \mu \text{mol/kg} \) i.p.) and (-)-nicotine (1.9 \( \mu \text{mol/kg} \) i.p.) were tested in rats after central treatment with the nAChR antagonist, chlorisondamine (10 \( \mu \text{g/rat i.c.v.} \)) (table 4). A cross-over design in which all animals received all systemic treatments (i.e., ABT-594, (-)-nicotine and saline) was used. Chlorisondamine was administered 5 days before the start of testing in the hot box. In control animals, ABT-594 and (-)-nicotine produced a significant antinociceptive effect in the hot box indicated by a significant overall drug effect \( F(2,56) = 33.083, P < .0001 \). A significant effect of chlorisondamine treatment was observed also \( F(1,56) = 19.511, P = .0006 \). In chlorisondamine-treated animals, the antinociceptive effect of ABT-594 was attenuated significantly but not blocked completely. In rats treated with (-)-nicotine, chlorisondamine pretreatment blocked the antinociceptive effect.

**Formalin test.** The effect of systemic (i.p.) ABT-594 treatment on both phase 1 and phase 2 of the formalin test was investigated. In control animals, a biphasic response pattern was observed during the course of the experiment (fig. 3). In both phase 1 and phase 2 (i.e., 30–50 min after formalin) of the protocol, ABT-594 at doses of 0.1 and 0.3 \( \mu \text{mol/kg} \) i.p. significantly attenuated nocifensive responding as indicated by an overall significant effect of treatment \( F(2,143) = 16.845, P = .0002 \). In a separate experiment, significant dose effects were observed in phase 2 of the formalin test after both i.p. \( F(3,28) = 15.167, P < .0001 \) and p.o. \( F(3,23) = 16.775, P < .0001 \) administration (fig. 4). Also, dose-dependent effects were observed after treatment with A-98593 \( F(3,27) = 8.140, P < .0005 \) (fig. 4). When ABT-594 (0.1 and 0.3 \( \mu \text{mol/kg} \) i.p.) was administered 10 min after the formalin injection, an overall treatment effect was found \( F(2,63) = 4.045, P = .022 \). Further analysis indicated a significant antinociceptive effect during phase 2 with 0.3 \( \mu \text{mol/kg} \) ABT-594 (fig. 5).

The antinociceptive effect of ABT-594 (0.3 \( \mu \text{mol/kg} \) i.p.) in phase 2 of the formalin test was attenuated, but not completely blocked by pretreatment with the noncompetitive nAChR antagonist, mecamylamine (1.5 \( \mu \text{mol/kg} \) i.p.) (table 5). Pretreatment with the opioid receptor antagonist, naltrexone (2.65 \( \mu \text{mol/kg} \) i.p.), did not affect the antinociceptive effect of ABT-594 in phase 2 of the formalin test (table 6). This dose of naltrexone was effective in completely attenuating the antinociceptive effect of morphine (data not shown). In rats previously treated with the nAChR antagonist, chlorisondamine (10 \( \mu \text{g/rat i.c.v.} \)), the antinociceptive effect of ABT-594 was blocked (table 7).

**Open-field activity.** The effects of acute i.p. administration of ABT-594 on open-field activity were examined. For vertical activity (fig. 6A), significant dose \( F(3,22) = 7.928, .0006 \).
TABLE 6
Effects in phase 2 of the formalin test of naltrexone pretreatment on the antinociceptive effect of ABT-594 (n = 4–12/group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Responsesa</th>
</tr>
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<tbody>
<tr>
<td>sal/sal</td>
<td>32.7 ± 7.1</td>
</tr>
<tr>
<td>ntx/sal</td>
<td>54.0 ± 9.9</td>
</tr>
<tr>
<td>sal/ABT-594</td>
<td>6.4 ± 4.0*</td>
</tr>
<tr>
<td>ntx/ABT-594</td>
<td>4.8 ± 3.4*</td>
</tr>
</tbody>
</table>

a Values are mean ± S.E.M.
* P < .05 vs. sal/sal.

TABLE 7
Effects in phase 2 of the formalin test of i.c.v. chlorisondamine pretreatment on the antinociceptive effect of ABT-594 (n = 4–12/group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Responsesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS/sal</td>
<td>81.0 ± 5.9</td>
</tr>
<tr>
<td>chlor/sal</td>
<td>75.7 ± 9.9</td>
</tr>
<tr>
<td>PBS/ABT-594</td>
<td>25.3 ± 7.0*</td>
</tr>
<tr>
<td>chlor/ABT-594</td>
<td>85.0 ± 6.8</td>
</tr>
</tbody>
</table>

a PBS, phosphate-buffered saline.
* Values are mean ± S.E.M.
* P < .05 vs. PBS/sal.

P = .0009] and time effects [F(3,66) = 46.954, P < .0001] were observed as well as a significant dose × time interaction [F(9,66) = 11.865, P < .0001]. Subsequent analysis of each of the 15-min time blocks showed a biphasic effect on vertical activity. During the first block, dose-dependent decreases in activity relative to the saline group were seen, with significant effects at the two higher doses (0.03 and 0.3 μmol/kg i.p.); whereas during the last block, the highest dose of ABT-594 (0.3 μmol/kg i.p.) increased activity relative to saline. Similar results were observed with horizontal activity (fig. 6B) in which a significant dose [F(3,22) = 8.001, P = .0009] and time effects [F(3,66) = 61.348, P < .0001] were observed

Fig. 3. ABT-594 treatment significantly reduces nocifensive responses in both phase 1 (0–10 min) and phase 2 (30–50 min) of the formalin test in rats. Values represent the mean ± S.E.M.; n = 6/group.

Fig. 4. Treatment with ABT-594 (i.p. and p.o.) and A-98593 (i.p.), the enantiomer of ABT-594, produces significant antinociceptive effects during phase 2 of the formalin test (n = 8/group).

Fig. 5. Administration of ABT-594 after the injection of formalin produces a significant antinociceptive effect during phase 2 of the formalin test (n = 22/group).

Table 5
Effects in phase 2 of the formalin test of mecamylamine pretreatment on the antinociceptive effect of ABT-594 (n = 4–12/group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Responsesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>sal/sal</td>
<td>41.0 ± 7.0</td>
</tr>
<tr>
<td>mec/sal</td>
<td>36.2 ± 8.9</td>
</tr>
<tr>
<td>sal/ABT-594</td>
<td>11.7 ± 2.9*</td>
</tr>
<tr>
<td>mec/ABT-594</td>
<td>25.5 ± 4.5</td>
</tr>
</tbody>
</table>

a Values are mean ± S.E.M.
* P < .05 vs. sal/sal.
as well as a significant dose × time interaction \( F(9,66) = 15.425, P < .0001 \).

The effects of acute oral administration of ABT-594 on open-field activity are shown in figure 6, C and D. For vertical activity (rearing) (fig. 6C), no significant dose effect was observed \( F(3,24) = 1.758, P = .182 \), but a significant effect of time \( F(3,72) = 34.268, P < .0001 \) and a significant dose × time interaction \( F(9,72) = 3.388, P = .002 \) were obtained. Subsequent analysis of each of the 15-min time blocks showed a decrease in vertical activity at 3 \( \mu \)mol/kg that was restricted to the first block. For horizontal activity (fig. 6D), a significant dose effect was not observed \( F(3,24) = 1.006, P = .407 \) but significant time \( F(3,72) = 47.335, P < .0001 \) and dose × time interaction \( F(9,72) = 5.518, P < .0001 \) effects were obtained. In contrast to the results with vertical activity, no decreases in horizontal activity were found when individual blocks were analyzed. Moreover, increased horizontal activity was noted with the two higher doses (0.3 and 3.0 \( \mu \)mol/kg) during the last two blocks. Thus, there was a decrease in rearing during the early portion of the session at the high dose but not at lower doses and increased horizontal activity at the two higher doses during the later part of the session.

To compare the effects of repeated and acute dosing with ABT-594 on open-field activity, three groups were given twice-daily i.p. injections for 5 days, and open-field behavior was assessed after the last injection. One group received saline for all injections, a second group received ABT-594 (0.3 \( \mu \)mol/kg) for all injections and a final group received saline for the first nine injections and ABT-594 (0.3 \( \mu \)mol/kg) for the tenth (last) injection. The first group served as the control group, the second group served as the repeated treatment group and the last group served as the acute treatment group. The effects of morphine (21 \( \mu \)mol/kg i.p.) also were assessed in this experiment by including a group that received ten injections of morphine and a group that received nine injections of saline and an injection of morphine just before being placed in the open field (i.e., repeated morphine and acute morphine groups, respectively). These results are presented in figure 7. For horizontal activity, treatment \( F(4,35) = 10.857, P < .0001 \) and time \( F(3,105) = 86.735, P < .0001 \) effects were significant, as was the treatment × time interaction \( F(12,105) = 5.879, P < .0001 \). Individual analysis of each 15-min block revealed that acute ABT-594 decreased horizontal activity during the first part of the session and increased activity during the latter part of the session, just as in the dose-response experiment. In rats that had received repeated exposure to ABT-594, however, horizontal activity was higher than control levels throughout the session. Acute morphine decreased horizontal activity early in the session, although the effect was only statistically significant during the second block. Rats previously exposed to repeated administration of morphine did not show this effect, however, and were actually more active than control rats later in the session. A comparable pattern of treatment effects was found for vertical activity (data not shown).

**Motor coordination.** The acute effects of ABT-594 on the edge balance test were statistically significant \( F(3,24) = 5.520, P = .005 \), with the 0.3 \( \mu \)mol/kg dose producing a significant reduction in performance and the 0.1 \( \mu \)mol/kg dose producing a nonsignificant trend in the same direction (data not shown). In a repeated dosing experiment, three groups received twice daily i.p. injections. One group received saline for all injections, a second group received ABT-594 (0.3 \( \mu \)mol/kg) for all injections and a final group received saline for the first nine injections and ABT-594 (0.3 \( \mu \)mol/kg) for the tenth (last) injection. Fifteen minutes after the tenth injection of the week, the rats were tested in the edge-balance procedure, and these data are presented in table 8. Analysis of the latencies to fall in the three groups revealed a significant treatment effect \( F(2,26) = 23.010, P < .0001 \). As in the dose-response experiment, animals treated acutely with 0.3 \( \mu \)mol/kg of ABT-594 fell off more quickly than controls. In contrast, rats that had received repeated injections of ABT-594 showed no deficit on this task. Immediately after the edge test, rectal temperatures were determined in these rats (table 8). There was a significant treatment effect \( F(2,26) = 12.142, P < .0001 \) with acute ABT-594 significantly reducing body temperature. There was no effect on body temperature in the rats who received repeated dosing with ABT-594.

**EEG.** Figure 8 shows that ABT-594 (0.03–0.3 \( \mu \)mol/kg i.p.) significantly \( F(7,31) = 8.486, P < .0016 \) lowered 1- to 50-Hz EEG amplitude (voltage). Examination of individual rat EEGs revealed that the onset of this effect was rapid; usually within 5 min after injection. Similarly, caffeine (50.0 \( \mu \)mol/kg i.p.), an adenosine antagonist with stimulant properties, significantly \( t(5) = 2.931, P = .03 \) lowered EEG amplitude. In contrast to both ABT-594 and caffeine, the opioid analgesic morphine (42.0 \( \mu \)mol/kg i.p.) significantly \( t(5) = -2.754, P = .04 \) increased 1- to 50-Hz amplitude, an effect consistent with increased expression of high-voltage EEG slow waves.

![Table 8](https://i.imgur.com/12345678.png)

**Table 8** Effects on performance of the edge task and body temperature (0.3 \( \mu \)mol/kg i.p.) in rats receiving repeated treatment (5 days b.i.d.) with either saline or ABT-594 (0.3 \( \mu \)mol/kg i.p.) (n = 6/group)

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>ABT-594</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge task (latency to fall [s])</td>
<td>120 ± 0</td>
<td>36.4 ± 10.5*</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.5 ± 0.2</td>
<td>36.7 ± 0.4*</td>
</tr>
</tbody>
</table>

* \( P < .05 \) vs. control. Control animals were dosed with saline repeatedly (5 days b.i.d.) and administered saline before the test.
duce antinociceptive activity by acting at multiple levels of the pain pathway. For example, ABT-594-induced antinociception may involve activation of descending monoaminergic pathways located in the brainstem such as the nucleus raphe magnus and locus ceruleus (Bitner et al., 1997; Curzon et al., 1997). In addition, ABT-594 may interact directly at the level of the spinal cord where it may inhibit the release of neurotransmitters such as substance P (Bannon et al., 1998) which is believed to be involved in the transmission of pain. Further work is needed to elucidate and characterize mechanisms that may be involved in the antinociceptive effect of ABT-594.

After acute systemic treatment at antinociceptive doses, ABT-594 produced a decrease in the 1- to 50-Hz spectral amplitude. This low-voltage, desynchronized cortical EEG pattern indicates cortical arousal or activation, and is typical of the effects of other nicotinic cholinergic modulators (Radek et al., 1996). It is also similar to patterns produced by dopamine agonists such as amphetamine, and adenosine antagonists such as caffeine (Bauer, 1987). A desynchronized EEG is expressed during waking periods of increased arousal and during REM sleep. The ABT-594-induced EEG desynchronization observed in the current study occurred during the waking state and did not indicate an increase in REM sleep. In contrast to ABT-594, large doses of opioid analgesics produce a high-voltage synchronized EEG characterized by slow wave and spindle bursts (Ferger and Kuschinsky, 1995). Opioids also block desynchronization evoked by stimulation of the reticular formation (Martin and Kay, 1977). The observed narcosis and hypokinesia produced by opioids is consistent with the effects that these drugs have on the EEG. The ability of ABT-594 to produce an EEG which corresponds to increased arousal differentiates this compound from opioid analgesics.

Discussion

In two well-characterized models of pain, the hot box (acute thermal pain) and the formalin test (persistent chemical pain), acute treatment with ABT-594 produced significant antinociceptive effects after both oral and i.p. routes of administration. In the hot box, ABT-594 was greater than 40-fold more potent than morphine. ABT-594 is also effective in mouse models of acute (i.e., hot plate) and persistent (i.e., abdominal constriction assay) pain after acute treatment (Decker et al., in press). Activity in both of these models of pain distinguishes ABT-594 from the nAChR ligand (-)-nicotine, which is inactive in models of persistent pain including the formalin test in rats (Bannon et al., 1998) as well as the abdominal constriction assay in mice (Decker et al., in press). The nAChR ligand, (±)-epibatidine, is effective in the hot box and formalin test (Nikkel et al., 1997), however there is poor separation between antinociceptive doses and toxic doses with (±)-epibatidine (Sullivan et al., 1994a, b). In the formalin test, ABT-594 was also effective when administered after the injection of formalin (noxious stimulus) which may be more clinically relevant in situations where analgesics are administered after frank tissue injury. Also, in a repeated dosing paradigm, ABT-594 maintained efficacy in the hot box which suggests that tolerance to the antinociceptive effects may not develop following repeated treatment with the nAChR ligand ABT-594.

The antinociceptive effects of ABT-594 (i.p.) in both the hot box and formalin tests were mediated by nAChRs as evidenced by attenuation of the antinociceptive effects of ABT-594 after administration of mecamylamine, a nAChR antagonist. Furthermore, blockade of central neuronal nAChRs with chlorisondamine treatment (i.c.v.) significantly attenuated the antinociceptive effects of ABT-594 and (−)-nicotine in the hot box and ABT-594 in the formalin test, which implicates a predominant role for central neuronal nAChRs. The lack of complete blockade of the antinociceptive effect of ABT-594 in the hot box by chlorisondamine treatment indicates that peripheral nAChRs may be involved at some level in the antinociceptive effects. In both the hot box and formalin test, ABT-594-induced antinociception was not altered by pretreatment with the opioid receptor antagonist naltrexone, which indicates that the antinociceptive effects of ABT-594 do not require activation of naltrexone-sensitive opioid receptors.

In general, initial work indicates that ABT-594 may produce antinociceptive activity by acting at multiple levels of the pain pathway. For example, ABT-594-induced antinociception may involve activation of descending monoaminergic pathways located in the brainstem such as the nucleus raphe magnus and locus ceruleus (Bitner et al., 1997; Curzon et al., 1997). In addition, ABT-594 may interact directly at the level of the spinal cord where it may inhibit the release of neurotransmitters such as substance P (Bannon et al., 1998) which is believed to be involved in the transmission of pain. Further work is needed to elucidate and characterize mechanisms that may be involved in the antinociceptive effect of ABT-594.

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At antinociceptive doses, acute dosing with ABT-594 significantly altered spontaneous locomotor activity after both oral and i.p. routes of administration. In general, acute treatment with ABT-594 produced a decrease in locomotor activity during the early portion of the test session (i.e., first 30 min) and produced an increase in activity (or lack of habituation) during the later portion of the test session (i.e., 30–60 min). Similar effects were observed after acute treatment with an antinociceptive dose of morphine. After i.p. administration, the effects on locomotor activity were clearly more pronounced relative to the effects observed after oral administration, which may reflect differences in pharmacokinetics (e.g., peak brain levels) between the two routes of administration. The biphasic change observed with locomotor activity after acute ABT-594 treatment was similar to that observed in naive animals after acute treatment with the nAChR ligand (−)-nicotine. In addition to effects on spontaneous locomotor activity, acute treatment with ABT-594 at a dose of 0.3 μmol/kg (i.p.) significantly impaired motor coordination as measured by the edge task. In mice, significant impairment of motor coordination (i.e., rotarod) was observed, but only at doses higher than the maximally effective antinociceptive doses (Decker et al., in press). However, in the current study, motor impairment was no longer observed after repeated dosing, whereas the antinociceptive efficacy was unaltered. Moreover, significant antinociception was observed in the hot box and the formalin test at a dose of 0.03 μmol/kg (i.p.) of ABT-594 that did not disrupt motor coordination as measured by the edge task.
One potential issue with the effects of ABT-594 on motor function is that these actions possibly interfere with the animal's ability to make a response (e.g., foot withdrawal in hot box and flinching behavior in formalin test) in the antinoceptive assays used in the current investigation. A compound that simply impairs the animal's ability to elicit a motor response would be identified as a potential analgesic agent based on the hot box and formalin test. In both of these assays, a significant reduction in responding is interpreted as an antinoceptive effect. In the present study, decreases in locomotor activity and impairment of motor coordination typically found after acute dosing with ABT-594 in drug-naive animals were not observed after an acute challenge dose of ABT-594 in rats treated repeatedly with ABT-594. In addition, reduction of body temperature observed after acute treatment with ABT-594 was not found in animals treated repeatedly with ABT-594. The apparent development of tolerance to the motor and temperature effects of ABT-594 was in contrast to the lack of tolerance to the antinoceptive effect. Antinociceptive activity of ABT-594 was sustained in rats maintained on a repeated dosing schedule with ABT-594 and then administered a challenge dose. Overall, the data with ABT-594 suggest that the effects on motor function and temperature can be dissociated from the effects on antinociception, and this may suggest involvement of different neuronal nAChR subtypes in these effects. Which neuronal nAChR subtypes that underlie the various effects of ABT-594 is not clear at this time. Also, the finding that oral administration attenuates the motor effects more than the antinociceptive effects suggests that some of the undesirable effects of ABT-594 may also be related to pharmacokinetic issues (i.e., high Cmax values with i.p. administration).

In conclusion, the current investigation indicates that the nAChR ligand ABT-594 is an effective antinociceptive agent with activity in preclinical models of acute thermal pain as well as persistent pain elicited by a chemical stimulus. The antinociceptive actions of ABT-594 in these models are mediated predominantly by central neuronal nAChRs, although involvement of peripheral nAChRs is not excluded entirely. The data also suggest that the antinociceptive effects of ABT-594 do not tolerate after repeated dosing, whereas the potential side effects of ABT-594 are reduced substantially with repeated administration. In addition, ABT-594 apparently does not have sedative-like effects on EEG which are characteristic of opioids like morphine. Further development of nAChR ligands such as ABT-594 for clinical use as analgesic agents ultimately could provide an alternative choice to opioids in pain management.

References


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