Negative Allosteric Modulation of Nicotinic Acetylcholine Receptors Blocks Nicotine Self-Administration in Rats

Ryan F. Yoshimura, Derk J. Hogenkamp, Wen Y. Li, Minhnam B. Tran, James D. Belluzzi, Edward R. Whittemore, Frances M. Leslie, and Kelvin W. Gee

Department of Pharmacology, School of Medicine, University of California, Irvine, California

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ABSTRACT

Drugs that antagonize nicotinic acetylcholine receptors (nAChRs) can be used to inhibit nicotine-induced behavior in both humans and animals. The aim of our experiments is to establish a proof-of-principle that antagonism of nAChRs by negative allosteric modulation can alter behavior in a relevant animal model of addiction, nicotine self-administration. We have identified a novel, negative allosteric modulator of nAChRs, UCI-30002 [N-(1,2,3,4-tetrahydro-1-naphthyl)-4-nitroaniline], with selectivity for the major neuronal nAChR subtypes over muscle-type nAChRs. After systemic administration, UCI-30002 significantly reduces nicotine self-administration in rats on both fixed ratio and progressive ratio schedules of reinforcement. The minimum effective dose that significantly alters nicotine self-administration corresponds to brain concentrations of UCI-30002 that produce at least 30% inhibition of the major neuronal nAChR subtypes measured in vitro. UCI-30002 has no effect on responding for food reinforcement in rats on either type of schedule, indicating that there is no effect on general responding or natural reward. UCI-30002 represents validation of the concept that negative allosteric modulators may have significant benefits as a strategy for treating nicotine addiction and encourages the development of subtype-selective modulators.

Tobacco use is responsible for 5 million deaths annually worldwide, and it is projected that half of all current smokers will die prematurely from illnesses related to their addiction (World Health Organization, 2005). In a 2000 United States poll, 70% of adult smokers wanted to quit, and 41% had tried to quit within the preceding year (Centers for Disease Control, 2002). Unfortunately, the prolonged abstinence rate of unaided smoking cessation can be as low as 3 to 5% (Hughes et al., 2004).

The action of nicotine at nAChRs lies at the core of nicotine addiction, and a variety of drugs represent clinical validation of nAChRs as a target for smoking cessation therapies. The three therapeutic strategies approved by the Food and Drug Administration for smoking cessation are the atypical antidepressant bupropion (Zyban). Varenicline and the nicotine replacement therapies are thought to partially replicate the activating or desensitizing actions of nicotine at nAChRs observed when smoking (Coe et al., 2005). Bupropion is an inhibitor of nAChRs (Slemmer et al., 2000) but also increases dopamine levels in the nucleus accumbens (Nomikos et al., 1989, 1992).

Ligands that act at nAChRs have been identified with competitive mechanisms of action such as varenicline (Coe et al., 2005) or erysodine (Mansbach et al., 2000) and with noncompetitive mechanisms such as bupropion (Slemmer et al., 2000) or crystal violet (Arias et al., 2006). Allosteric modulators are a class of noncompetitive ligands that act on a separate, distinct site from the agonist (orthosteric) binding site and exert their effect either by changing agonist-receptor affinity or the intrinsic efficacy of the receptor (Ehlert, 2005). The general mechanism of allosteric modulation has been well established clinically by the benzodiazepines, which are positive allosteric modulators of GABA<sub>A</sub> receptors. Likewise, in the nicotinic field, positive allosteric modulators such as galantamine are in clinical use (Samochcki et al., 2003). Our interest is in demonstrating the behavioral relevance of negative allosteric modulation of nAChRs for nicotine addiction.

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Abbreviations: DHβE, dihydro-β-erythroidine; nACHR, nicotinic acetylcholine receptor; UCI-30002, N-(1,2,3,4-tetrahydro-1-naphthyl)-4-nitroaniline; NMDA, N-methyl-D-aspartate; PEG, polyethylene glycol 400; DSW, 5% dextrose in water; DMSO, dimethyl sulfoxide; FR, fixed ratio; TO20, 20-s dark time-out period; BP, break point; ANOVA, analysis of variance.
Inhibition of nAChR function has shown behavioral efficacy in animal models of nicotine addiction. The nonselective antagonist mecamylamine, but not the peripherally restricted hexamethonium, blocks self-administration in rats with limited (Corrigall and Coen, 1989) or extended (DeNoble and Mele, 2006) access to nicotine. This observation indicates the involvement of nAChRs in the central nervous system. The α4β2 subtype-selective competitive antagonist dihydro-β-erythroidine (DHβE) also inhibits nicotine self-administration in rats (Corrigall et al., 1994), suggesting a prominent role of α4β2-containing nAChRs in nicotine addiction. These pharmacological studies have been more recently supported by molecular studies implicating the importance of β2-containing (Picciotto et al., 1998; Maskos et al., 2005) and α4-containing (Tapper et al., 2004) nAChRs in behavioral responding for nicotine rewards.

Our laboratory has previously identified a series of positive allosteric modulators of GABA_{A} receptors (Johnstone et al., 2004). Using the rationale that allosteric sites may be conserved between GABA_{A} receptors and nAChRs because they belong to the same superfamily of ligand-gated ion channels, we screened our library of allosteric modulators at α4β2 nAChRs in search of negative efficacy modulators. We identified UCI-30002 as a novel, nonselective negative allosteric modulator of nAChRs that shows voltage independence. UCI-30002 blocks nicotine self-administration in rats at brain concentrations consistent with nAChR inhibition. Importantly, UCI-30002 does not operate by reducing general reward responding because there is no effect on food reward. Thus, UCI-30002 represents the proof-of-principle confirmation of the utility of negative allosteric modulation of nAChRs in reducing nicotine behavior. Continued development of selective negative allosteric modulators will provide another strategy for clinical studies with defined benefits to facilitate smoking cessation and will also serve as tools to differentiate the role(s) of distinct nAChR subtypes in nicotine-mediated behavior.

Materials and Methods

Subjects. Adult male Sprague-Dawley rats weighed approximately 300 g on arrival from Charles River Laboratories (Wilmington, MA). Adult male Swiss-Webster or non-Swiss albino (CF-1 outbred) mice weighed approximately 25 g on arrival from Harlan (San Diego, CA). Animals were group housed with access to food and water ad libitum. All animals were maintained on a 12-h light/dark cycle, and all experiments were run during the light cycle. All experimental procedures followed guidelines approved by the University of California Irvine Institutional Animal Care and Use Committee and were consistent with federal guidelines.

Drugs. UCI-30002 was synthesized in house as detailed below. Nicotine hydrogen tartrate, GABA, NMDA, glycine, and mecamylamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). For in vivo experimentation, UCI-30002 was solubilized in a vehicle of a 4:1 ratio of polyethylene glycol 400 (PEG) to 5% D5W. Nicotine-Induced Seizures. Adult male non-Swiss albino mice were pretreated with either UCI-30002 (10 mg/kg i.p., 1 ml/kg; n = 10 per group) or the PEG/D5W vehicle 15 min before nicotine injection (4 mg/kg free base s.c., 1 ml/kg). The animals were then monitored for the next 30 min for the incidence of clonic and tonic seizures and scored as having seizures after one episode of clonic or tonic activity.

Pharmacokinetics. Adult male Sprague-Dawley rats were anesthetized by halothane inhalation at different time points following administration of UCI-30002 (n = 5 per time point). Blood was drawn by cardiac puncture, and the brain was perfused with sterile saline. Plasma and brain samples were solvent extracted and analyzed by high-performance liquid chromatography to determine compound concentration and to calculate the compound half-life in the animal body.

Locomotor Activity. Adult male Swiss-Webster mice were pretreated with saline (n = 6), the PEG/D5W vehicle (n = 7), or UCI-30002 (10 mg/kg i.p., 1 ml/kg; n = 7) in a darkened room 30 min before testing. Locomotor activity was monitored in an enclosed activity chamber (26 × 26 × 39 cm) by a computer using TruScan 2.01 software (Coulbourn Instruments, Allentown, PA).

Nicotine Self-Administration. Adult male Sprague-Dawley rats were food restricted (5–10 g chow per day) to ~85% of their initial body weight and trained to nose poke in a standard two-nose poke operant chamber (Med Associates, Inc., St. Albans, VT) on a fixed ratio (FR) 1 schedule for a 45-mg food pellet. A response at the reinforced nose poke resulted in a brief illumination of a paired cue light (~1 s) above the nose poke and a food pellet dropped in a bin. Responses at the nonreinforced nose poke were recorded but had no consequence. The food training sessions continued daily, and the rat was considered to have acquired the task when it reached the 100 reinforced responses within the 20-min session.

After drying over Na$_2$SO$_4$, the mixture was filtered, and the solvent was removed under reduced pressure. The resulting yellow-orange oil was triturated with 100 ml of hexanes giving 2.35 g of the desired product. Recrystallization from EtOH gave a yellow solid, m.p. 131.5 to 133°C. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.11 (d, 2H, J = 9.2 Hz), 7.31–7.15 (m, 4H), 6.60 (d, 2H, J = 9.2 Hz), 4.74 (br s, 2H), 2.91–2.76 (m, 2H), 2.05–1.98 (m, 2H), 1.92–1.84 (m, 2H). The structure of UCI-30002 is illustrated as an inset in Fig. 1.
Construction, implantation, and maintenance of the i.v. catheter were as described previously (Belluzzi et al., 2005). After catheterization, the animals were individually housed. The catheters were flushed daily with a heparinized saline solution (4 U of heparin in 200 μl of saline) to maintain patency. Animals were tested weekly with propofol to confirm catheter patency, and data from any rats that did not demonstrate rapid anesthesia were discarded.

After catheter implantation, the rats were returned to a light food restriction (20 g chow per day) to maintain a healthy, adult body weight (350–450 g). Nicotine self-administration was initially established on a FR1 schedule. Each reinforced response resulted in a single i.v. nicotine infusion of 0.03 mg/kg (free base; 20 μl over 1 s) through the jugular catheter, a momentary activation of the paired cue light above the reinforced nose poke (1 s), after which the house light was shut off for a 20-s dark time-out period (TO20). During the timeout period, any responses by the animal had no consequence. The nicotine self-administration session ended after 2 hours or a maximum of 100 reinforced responses. The schedule was increased as the animal reached a stable baseline from FR1 to FR2, then from FR2 to FR5. Criteria for a stable baseline was at least 2 consecutive days of reinforced responses within 15% of each other (minimum of 15 reinforced responses) and a ratio of reinforced to nonreinforced responses that was greater than approximately 2:1. For example, a rat with reinforced responses of 25 and 30 on consecutive days would be stable, whereas 23 and 32 responses would not be stable. Animals stably self-administering at FR5 were then injected with either the PEG/D5W vehicle or UCI-30002 (3 or 10 mg/kg) 30 min before the session (for saline or mecamylamine, 15 min prior). The number of reinforced responses was compared with the previous 2-day baseline to determine whether the drug had an effect on reinforced responding. The number of nonreinforced responses was used as a control for nonspecific effects of the drug. Each rat received all treatments, and the order of administration of drug or vehicle for any single rat was determined randomly with approximately half receiving drug first. After animals had been self-administering nicotine at FR5 for ~30 days, they were tested on a progressive ratio schedule. The break point (BP) schedule was determined using the formula: BP = 56^{1g} \times 0.2^{1d} - 5 (Richardson and Roberts, 1996). For example, nicotine was self-administered to the rats using the series 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, and so forth. Animals were given 30 min to reach each consecutive BP or an overall time limit of 4 h, after which the session was terminated. Animals were treated with the same counterbalanced design used in the fixed ratio experiment. The animals were allowed to re-establish a nicotine baseline at the FR5 schedule before the next progressive ratio test. A progressive ratio session with no injection was used as the baseline for vehicle and drug comparisons for each animal.

**Food Administration.** Adult male Sprague-Dawley rats were trained to respond for food as in the nicotine studies and followed the same restricted diet. Animals then were trained to respond to in-
creasing schedule and timeout requirements (FR1TO10, FR1TO20, FR2TO20, FR5TO20) with a maximum of 100 reinforcers in 60-min sessions. Once at FR5, the maximum number of reinforcers was removed. Using the same baseline criteria as described in the nicotine studies, animals stably administering at FR5TO20 were tested with either PEG/D5W or UCI-30002. The animals were then tested on the same progressive ratio schedule as in the nicotine studies with an overall time limit of 2 h.

Statistics. The dose response of inhibition was evaluated by non-linear regression using Prism 4.03 (GraphPad Software, Inc., San Diego, CA) to determine IC50 or EC50 values. Statistics for the electrophysiological studies were determined by one-way ANOVA with post hoc analyses using Bonferroni’s multiple comparison test. Statistics for the nicotine seizure studies were determined by Fisher-Yates exact probability test. Statistics for the locomotor studies were determined by repeated measures (time) two-way ANOVA with post hoc analyses using Bonferroni’s multiple comparison test. Statistics for the self-administration studies were determined by paired Student’s t test or by one-way ANOVA with post hoc analyses using Bonferroni’s multiple comparison test as appropriate. The criterion for statistical significance is 0.05.

Results

Inhibition of nAChRs by UCI-30002. The activity of UCI-30002 at various nAChR subtypes was measured by electrophysiology in Xenopus oocytes expressing human (α4β2, α7), rat (α3β4), and mouse (α1β1δε) nAChRs (Fig. 1). UCI-30002 evoked concentration-dependent inhibition of the three major neuronal subtypes (α4β2, α7, α3β4), with IC50 values of 1.3 ± 0.2, 2.0 ± 0.2, and 4.5 ± 1.0 μM (mean ± S.E.M.), respectively. UCI-30002 evoked full blockade of α7 and α3β4 subtypes but only partial blockade (~80%) of α4β2. UCI-30002 was substantially less potent for blockade of the adult muscle-type nAChR (α1β1δε) with an apparent IC50 greater than 30 μM. The maximal inhibition of the adult muscle-type nAChR by UCI-30002 was unattainable due to insolvency at concentrations greater than 30 μM. At all subtypes, the inhibitory effects of UCI-30002 were reversed rapidly following washout. A concentration of 10 μM UCI-30002 was inactive at human α1β2γ2 GABAA receptors and inhibited NMDA receptors by 13% (Fig. 1, inset).

Electrophysiological Characterization of UCI-30002. The extent of inhibition of α4β2 nAChRs by 3 μM UCI-30002 was independent of agonist concentration, indicating a non-competitive mechanism (F3,11 = 0.018, N.S.; Fig. 2A). Likewise, the inhibition of α4β2 nAChRs by 3 μM UCI-30002 was independent of membrane potential, indicating a voltage-independent mechanism (F3,12 = 0.129, N.S.; Fig. 2B).

Effects of UCI-30002 on Nicotine-Induced Seizures. A nicotine dose of 4 mg/kg s.c. will induce clonic/tonic seizures in 99% of adult male non-Swiss albino mice (ED99). In the PEG/D5W vehicle treatment group (n = 10), 100% of the mice displayed clonic/tonic seizures following nicotine. In contrast, following an i.p. injection of 10 mg/kg UCI-30002 (n = 10), only 30% of the mice in this treatment group showed clonic/tonic seizures following nicotine (data not shown). The Fisher-Yates exact probability test demonstrated a statistically significant effect of UCI-30002 on nicotine-induced seizures (P = 0.003).

Pharmacokinetics of UCI-30002. The elimination half-life of UCI-30002 in the plasma was estimated at 3 h following a 10 mg/kg i.p. dose in adult male Sprague-Dawley rats (Fig. 3). The brain concentration of UCI-30002 dropped to

![Fig. 2. A, UCI-30002 at a concentration of 3 μM maintained an equal level of inhibition as nicotine concentrations rose in oocytes expressing human α4β2 nAChRs (n = 3–4). B, UCI-30002 at a concentration of 3 μM maintained an equal level of inhibition of nicotine currents as the membrane potential changed in oocytes expressing human α4β2 nAChRs (n = 4). Data are presented as a percentage of the nicotine control current. There was no statistical significance as determined by one-way ANOVA with Bonferroni’s post hoc comparison.](image_url)

![Fig. 3. UCI-30002 was bioavailable in the brain following a single 10 mg/kg i.p. injection in adult male Sprague-Dawley rats (n = 5 per point). Approximate concentrations in the brain were at least 1 μM for 4 h following injection. UCI-30002 had a plasma half-life of ~3 h.](image_url)
levels near 1 μM for the first 4 h following treatment.

Effects of UCI-30002 on Locomotor Activity. Intrapерitoneal injection of adult male Swiss-Webster mice with saline, the PEG/D5W vehicle, or 10 mg/kg UCI-30002 had no significant effect of treatment on the distance traveled (F(2,17) = 0.095, N.S.; Fig. 4A), movement time (F(2,17) = 0.113, N.S.; Fig. 4B), or number of moves (F(2,17) = 0.835, N.S.; data not shown) in any of the 15-min time bins over the 2-h observation. As expected, there was a significant interaction of time on distance traveled (F(2, 119) = 55.30, P < 0.0001), movement time (F(2, 119) = 13.74, P < 0.0001), and the number of moves (F(2, 119) = 6.265, P < 0.0001).

Effects of UCI-30002 on a Fixed Ratio Schedule of Self-Administration. UCI-30002 reduced reinforced responding for nicotine on an FR5 schedule in adult male Sprague-Dawley rats (F(2, 12) = 8.352, P = 0.002; Fig. 5). Average baseline responding (mean ± S.E.M.) across all animals was 32.7 ± 1.3 at the reinforced nose poke and 12.8 ± 1.3 at the non-reinforced nose poke. Pretreatment with the PEG/D5W vehicle i.p. had no effect on responding for nicotine on an FR5 schedule compared with the baseline. Bonferroni’s post hoc analysis showed that pretreatment with 3 mg/kg i.p. UCI-30002 was not significantly different from the PEG/D5W vehicle (P > 0.05). Pretreatment with 10 mg/kg i.p. UCI-30002 significantly attenuated the number of nicotine infusions compared with the PEG/D5W vehicle (P < 0.01) and the 3 mg/kg dose (P < 0.01). The dose of 10 mg/kg UCI-30002 showed no differences in the inhibition of reinforced responding between the 1st and 2nd h of each session (t = 0.264, df = 9, P > 0.05; data not shown). The dose of 10 mg/kg UCI-30002 did not reduce the number of responses at the inactive lever (F(2, 21) = 1.551, N.S.; data not shown). Due to limitations in solubility of UCI-30002, higher doses were not tested. By comparison, pretreatment with 3 mg/kg s.c. mecamylamine significantly reduced reinforced responding for nicotine compared with saline (t = 4.586, df = 6, P < 0.01), whereas responding at the inactive lever was not significantly affected (t = 2.254, df = 6, P > 0.05).

A dose of 10 mg/kg i.p. UCI-30002 had no effect on reinforced responding for food rewards on a fixed ratio schedule in adult male Sprague-Dawley rats compared with the PEG/D5W vehicle (t = 1.446, df = 7, P > 0.05; Fig. 5, inset). For the FR5 study, the average baseline responding (mean ± S.E.M.) across all animals was 149.5 ± 2.1 at the reinforced nose poke and 17.2 ± 2.0 at the non-reinforced nose poke.

Effects of UCI-30002 on a Progressive Ratio Schedule of Self-Administration. UCI-30002 reduced reinforced responding for nicotine on a progressive ratio schedule in adult male Sprague-Dawley rats (F(2, 12) = 8.628, P = 0.005; Fig. 6). Bonferroni’s post hoc analysis showed that pretreatment with the PEG/D5W vehicle i.p. had no effect on responding for nicotine compared with untreated sessions (P > 0.05). Pretreatment with 10 mg/kg i.p. UCI-30002 significantly decreased the break point for nicotine compared with both untreated sessions (P < 0.01) and the PEG/D5W vehicle (P < 0.05). UCI-30002 did not affect responding at the inactive lever (F(2, 12) = 1.219, N.S.; data not shown). Limitations in solubility of UCI-30002 prevented testing of higher doses.

UCI-30002 also did not have a significant effect on reinforced responding for food rewards on a progressive ratio schedule compared with the PEG/D5W vehicle (t = 1.825, df = 7, P > 0.05; Fig. 6, inset). The number break points attained in untreated sessions using either food (21.3 ± 0.7) or nicotine (16.7 ± 2.1) was not significantly different (t = 2.155, df = 13, P > 0.05).

Discussion

We have identified UCI-30002 as a novel inhibitor of neuronal nAChRs. UCI-30002 inhibits neuronal, but not muscle-type, nAChRs. UCI-30002 displays properties of a negative allosteric modulator in that it is reversible, noncompetitive, and voltage-independent at α4β2 nAChRs in oocyte electrophysiology. UCI-30002 readily penetrates the blood-brain barrier, blocks nicotine-induced seizures in mice, and inhibits nicotine self-administration in rats.

The mechanism by which ligands interact with receptors can vary widely. In the nicotinic field, competitive antagonists (e.g., DHβE, methyllycocainsine) compete with nicotine or ACh to bind to the endogenous agonist (orthosteric) site on the receptor. Channel blockers (e.g., mecamylamine) are a class of noncompetitive ligands that do not compete for the orthosteric site but instead bind within the ion channel pore to physically block channel function. Many channel blockers exhibit voltage dependence due to interactions with the
membrane electrical field. Allosteric modulators are a class of noncompetitive ligands that act at a site distinct from the orthosteric site and from voltage-dependent channel sites. Negative allosteric modulation provides several advantages over competitive antagonism such as independence from agonist concentrations, an inherent ceiling effect, and probe-dependent efficacy (Kenakin, 2004). A negative allosteric modulator will inhibit the receptor by the same percentage at any agonist concentration and will have a maximal inhibition at saturation. It can also potentially alter receptor sensitivity to some orthosteric ligands while not affecting others.

Molecular manipulations have identified a wealth of information regarding the potential roles of specific proteins and pathways in nicotine addiction. However, more diverse pharmacological tools are needed to support the interpretations made in intact animals. UCI-30002 represents a novel pharmacological tool, an inhibitor of nAChRs with an allosteric and voltage-independent mechanism of action. Use of allosteric modulators such as UCI-30002 across a wide range of experimental paradigms will allow us to observe the effects of pharmacological blockade of nAChRs independent of agonist concentration and membrane potential in both in vitro and in vivo settings. Although identification of receptor subtype-selective allosteric modulators of nAChRs will represent a class of compounds with a significantly different mechanism from the conventional orthosteric ligands currently used in animal experimentation, such as DHβE and methyllycaconitine, valuable information can still be gained from nonselective compounds like UCI-30002.

Nicotine-induced seizures are potentially mediated at least...
in part by the α3β4 (Salas et al., 2004), α7 (Damaj et al., 1999), and α4β2 (Fonck et al., 2005) nAChR subtypes. The ability of UCI-30002 to block nicotine-induced seizures provides the initial evidence that it is present at these central nAChRs of interest. This is further confirmed by pharmacokinetic analysis that shows brain levels of at least 1 μM for 4 h following i.p. administration.

UCI-30002 provides proof-of-principle that nicotine self-administration is amenable to allosteric modulation of nAChRs. The inhibition of responding for nicotine at both fixed ratio and progressive ratio schedules is in agreement with the effects seen with competitive and noncompetitive nicotinic antagonists of nAChRs (Mansbach et al., 2000). Significantly, the effect on nicotine self-administration can be correlated to brain levels of UCI-30002 and inhibition of neuronal nAChRs with no effect on locomotor activity or food reward. The lack of an effect by UCI-30002 on reducing food administration at a fixed ratio schedule can potentially be attributed to a resistance effect due to food being more rewarding to the animal. However, the progressive ratio data indicates that there is no significant difference in the break point attained with either 0.03 mg/kg injections of nicotine or 45-mg food pellets, suggesting that the reinforcing efficacy of these rewards is roughly equivalent and that the assay should be able to detect alterations in reward value. The sensitivity of these conditions is supported by literature showing changes in break point for food reward using similar paradigms (Bruijnzeel and Markou, 2003).

That UCI-30002 maintains the same level of inhibition of reinforced responding in the 1st and 2nd h of nicotine self-administration suggests that a dose that corresponds to nAChR inhibition as low as 30% in vitro can sustain a robust behavioral response. Furthermore, the 3 mg/kg dose of UCI-30002 showed no behavioral effect, indicating that at some
point less than 30%, inhibition of nAChRs was ineffective. This gives us a starting point from which to evaluate the minimum efficacy of more subtype-selective allosteric modulators and, thus, an opportunity to quantitatively define the roles that these subtypes play in responding for nicotine. The behavioral inhibition seen with UCI-3002 appears independent of activity at GABA\textsubscript{A} or NMDA receptors because the brain concentration reached at the effective dose has no significant effect at either receptor type. It is possible that the reduction of behavioral responding in the nicotine self-administration experiments could be due to induction of acute nicotine withdrawal. The potential for a negative allosteric modulator to induce nicotine withdrawal will need to be evaluated in future experiments.

UCI-3002 is not the first reported negative allosteric modulator of nAChRs. Bupropion has been characterized as a noncompetitive and voltage-independent inhibitor of \(\alpha4\beta2\) nAChRs with an IC\textsubscript{50} of 8 \(\mu\text{M}\) (Slemmer et al., 2000). Bupropion has shown efficacy against nicotine addiction in rats and humans, but the precise mechanism for this efficacy is unclear (Hayford et al., 1999). The minimum effective dose of bupropion on nicotine self-administration in rats (Bruijnzee and Markou, 2003), and the corresponding brain concentrations (Suckow et al., 1986) are inconsistent with functional inhibition of nAChRs in vivo. The concentrations appear to far exceed what is necessary for \(\alpha4\beta2\) nAChR inhibition and are more on par with the doses required to see significant increases in extracellular dopamine in the nucleus accumbens (Nomikos et al., 1989). Thus, the accumulation of monoamines under these conditions may be the dominant factor in the ability of bupropion to inhibit nicotine self-administration in rats; therefore, bupropion can operate independently of \(\alpha4\beta2\) nAChR blockade. Monoamine accumulation as the primary mechanism is further supported by evidence that high doses of bupropion are self-administered in rats (Tell et al., 1997), a behavior not normally associated with nicotinic antagonism. Bruijnzee and Markou (2003) also report that bupropion does not have an effect on a progressive ratio schedule of nicotine self-administration but significantly increases the number of break points achieved for food reward. This also suggests that dopamine accumulation may be of greater importance than nAChR inhibition in the behavioral response to bupropion. In contrast, the effects of UCI-3002 appear highly correlated with nAChR inhibition, suggesting that it fits an appropriate profile for evaluating the behavioral effects of allosteric antagonism at nAChRs.

Another distinguishing property of UCI-3002 is that the inhibition of nAChRs does not exhibit voltage dependence. Many of the noncompetitive compounds described in the literature such as mecamylamine (Varanda et al., 1985; Papke et al., 2001) or crystal violet (Arias et al., 2006) inhibit neuronal nAChRs by a voltage-dependent mechanism, suggesting that they act as channel blockers. The inhibitory efficacy for voltage-dependent blockers will change as a function of the membrane potential. In the intact animal, cortical neurons experience subthreshold fluctuations in membrane potential in correlation with the level of network activity (Destexhe et al., 2003). Thus, a voltage-dependent inhibitor will block contributions from the receptors less efficiently in neurons that have lower membrane potentials. In contrast, a negative allosteric modulator would inhibit each receptor equally, independent of the membrane potential. Whether this holds any clinical relevance remains to be seen, but the evidence that UCI-3002 blocks nicotine self-administration indicates that a voltage-dependent mechanism is not necessary to affect the behavioral output. The mechanistic differences between voltage-dependent and -independent allosteric modulators will be highlighted as more comparative experimental data are gathered.

We hypothesize that UCI-3002 is hitting a common allosteric site at each of the neuronal nAChR subtypes tested. This allosteric site appears to have little functional contribution on the adult muscle-type nAChR tested. However, further studies will be needed to determine whether the allosteric site is altered on this subtype or if the site is conserved but has a minimal impact on the intrinsic efficacy of the agonist-receptor complex. Although we have provided evidence that UCI-3002 has an allosteric mechanism of action at \(\alpha4\beta2\) nAChRs, specific tests for competition and voltage dependence at all of the receptor families and subtypes remain to be done to rule out the possibility of different mechanisms of action at these receptors. Further studies should also be done to rule out alternate mechanisms of action. These include tests on dopamine receptors, dopamine transporters, and muscarinic acetylcholine receptors. Regardless of these possibilities, we have demonstrated that UCI-3002 is an allosteric modulator of \(\alpha4\beta2\) nAChRs and that at relevant brain levels in rats, it inhibits a behavior related to these receptors, nicotine self-administration.

UCI-3002 is one of many compounds that inhibited \(\alpha4\beta2\) nAChRs during a screening of compounds related to novel GABA\textsubscript{A} modulators. UCI-3002 is used in these proof-of-principle studies because it is reasonably potent and efficacious, is present in the brain, and has no overt toxicity. However, the structure of UCI-3002 prevents it from being a compelling clinical candidate for drug development. UCI-3002 is a nitroaryl compound that may be problematic because, under aerobic conditions, one of the biotransformation pathways of these compounds may be reduction of the nitro group in different subcellular organelles (e.g., microsomes, mitochondria, and cytosol) where a one-electron reduction can provoke the formation of a superoxide anion and a hydroxyl radical (Moreno et al., 1984; Docampo et al., 1988; Iwata et al., 1992). Generation of reactive oxygen species without their efficient removal can lead to toxicity resulting from the oxidative modification of proteins, lipids, and DNA (Neuzil et al., 1993). Nevertheless, although UCI-3002 will not be advanced as a clinical candidate with human utility, it has all the properties of an intriguing tool for the analysis of negative allosteric modulation of nAChR function. Additionally, we have begun work on more drug-like structural templates that may yield receptor subtype-selective allosteric modulators.

The neural adaptations that occur during the acquisition, maintenance, and extinguishing of nicotine behaviors are complex and not yet fully understood. The lack of a variety of well characterized pharmacological tools prevents the accurate exploration of the subtleties involved in the chain of events that begin when nicotine binds to nAChRs. This subsequently impedes the interpretation of the action of nicotine on the scale of individual cells, on populations of neurons, and, ultimately, in the whole animal. UCI-3002 represents a novel tool for pharmacological modification of nAChRs via an allosteric site in a voltage-independent manner. The con-
continued development of selective negative allosteric modulators of nAChRs will allow a more detailed pharmacological characterization of the contributions of nicotine and endogenous ACh, as well as the subtypes of nAChRs that contribute to the formation, persistence, and relapse liability of nicotine addiction.

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References


Address correspondence to: Dr. Kelvin W. Gee, Department of Pharmacology, University of California, Medical Surge I, Room 110C, Irvine, CA 92697.

E-mail: kwgee@uci.edu