Effects of Nicotinic Acetylcholine Receptor Agonists in Assays of Acute Pain-Stimulated and Pain-Depressed Behaviors in Rats

Kelen C. Freitas, F. Ivy Carroll, and S. Stevens Negus

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia; and Research Triangle Institute, Research Triangle Park, North Carolina

Received June 12, 2015; accepted September 8, 2015

ABSTRACT

Agonists at nicotinic acetylcholine receptors (nAChRs) constitute one drug class being evaluated as candidate analgesics. Previous preclinical studies have implicated $\alpha 4\beta 2$ and $\alpha 7$ nAChRs as potential mediators of the antinociceptive effects of (–)-nicotine hydrogen tartrate (nicotine) and other nAChR agonists; however, these studies have relied exclusively on measures of pain-stimulated behavior, which can be defined as behaviors that increase in frequency, rate, or intensity after presentation of a noxious stimulus. Pain is also associated with depression of many behaviors, and drug effects can differ in assays of pain-stimulated versus pain-depressed behavior. Accordingly, this study compared the effects of nicotine, the selective $\alpha 4/6\beta 2$ agonist 5-(123I)iodo-3-[2(S)-2-azetidylmethoxy]pyridine (5-I-A-85380), and the selective $\alpha 7$ agonist *N*-(3*R*)-1-azabicyclo(2.2.2)oct-3-yl-4-chlorobenzamide in assays of pain-stimulated and pain-depressed behavior in male Sprague-Dawley rats. Intraperitoneal injection of dilute lactic acid served as an acute noxious stimulus to either

stimulate a stretching response or depress the operant responding, which is maintained by electrical brain stimulation in an intracranial self-stimulation (ICSS) procedure. Nicotine produced a dose-dependent, time-dependent, and mecamylamine-reversible blockade of both acid-stimulated stretching and acid-induced depression of ICSS. 5-I-A-85380 also blocked both acid-stimulated stretching and acid-induced depression of ICSS, whereas *N*-(3*R*)-1-azabicyclo(2.2.2)oct-3-yl-4-chlorobenzamide produced no effect in either procedure. Both nicotine and 5-I-A-85380 were ≥ 10 -fold more potent in blocking the acid-induced depression of ICSS than in blocking the acid-induced stimulation of stretching. These results suggest that stimulation of $\alpha 4\beta 2$ and/or $\alpha 6\beta 2$ nAChRs may be especially effective to alleviate the signs of pain-related behavioral depression in rats; however, non-selective behavioral effects may contribute to apparent antinociception.

Introduction

Agonists at nicotinic acetylcholine receptors (nAChRs) constitute one drug class being evaluated as candidate analgesics for the treatment of pain. Antinociceptive effects of (–)-nicotine hydrogen tartrate (nicotine), a relatively non-selective nAChR agonist, have been demonstrated in pre-clinical and clinical studies (Tripathi et al., 1982; Damaj et al., 1994; Ditre et al., 2011; Nirogi et al., 2013), although the therapeutic window is narrow (Greiff et al., 1993; Weingarten et al., 2008; Mishriky and Habib, 2014). Prevailing evidence suggests that nicotine antinociception is mediated at least in part by $\alpha 4\beta 2$ and/or $\alpha 6\beta 2$ nAChRs (i.e., nAChRs containing $\alpha 4$ and/or $\alpha 6$ subunits in addition to $\beta 2$ subunits, hereafter identified as $\alpha 4/6\beta 2$ nAChRs). For example, nicotine, epibatidine, and ABT-594 all produce antinociception in preclinical models (Flores, 2000) and function as potent and efficacious $\alpha 4\beta 2$ agonists (Donnelly-Roberts et al., 1998). Moreover, nicotine antinociception in rodents can be attenuated by the

nonselective nAChR antagonist mecamylamine and the more selective $\alpha 4/6\beta 2$ antagonist dihydro- β -erythroidine (Cooley et al., 1990; Iwamoto, 1991; Damaj et al., 1995; Abidin et al., 2006) or by genetic deletion of $\alpha 4$ or $\beta 2$ nAChR subunits (Marubio et al., 1999). Lastly, a recent study presented evidence to suggest an important role for $\alpha 6^*$ receptors in mediating both nociception and nicotine antinociception in mice (Wieskopf et al., 2015).

In addition to $\alpha 4/6\beta 2$, the $\alpha 7$ nAChR subtype may also contribute to the antinociceptive effects of nicotine or other nAChR agonists. In vitro studies of both receptor binding (Kem et al., 1997; Jensen et al., 2003) and functional activity (Gerzanich et al., 1995; Eaton et al., 2003) suggest that nicotine is approximately 100-fold selective for $\alpha 4\beta 2$ versus $\alpha 7$ nAChRs, and the selective $\alpha 7$ antagonist methyllycaconitine (MLA) failed to block thermal antinociception by nicotine in rats (Rao et al., 1996). Nonetheless, MLA did antagonize the antinociceptive effects of intrathecal nicotine in spinal nerve-ligated rats (Young et al., 2008), and a negative allosteric modulator of $\alpha 7$ nAChRs (*meta*-chlorophenylguanidine) antagonized nicotine antinociception in a rat tail-flick procedure (Dukat et al., 2010). Moreover, $\alpha 7$ nAChR selective agonists produced antinociception in some rodent models of acute and

This research was supported by the National Institutes of Health National Institute of Neurological Disorders and Stroke [Grant R01 NS070715].
dx.doi.org/10.1124/jpet.115.226803.

ABBREVIATIONS: ANOVA, analysis of variance; 5-I-A-85380, 5-(123I)iodo-3-[2(S)-2-azetidylmethoxy]pyridine; ICSS, intracranial self-stimulation; MCR, maximum control rate; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; nicotine, (–)-nicotine hydrogen tartrate; PNU 282987, *N*-(3*R*)-1-azabicyclo(2.2.2)oct-3-yl-4-chlorobenzamide.

inflammatory pain (Damaj et al., 1998, 2000; Wang et al., 2005; Gao et al., 2010), and $\alpha 7$ positive allosteric modulators produced antinociception in mouse models of inflammatory and neuropathic pain (Freitas, et al., 2013a,b).

Preclinical studies to evaluate the antinociceptive effects of nicotine and other nAChR agonists have relied exclusively on assays of pain-stimulated behavior, which measure behaviors (e.g., withdrawal reflexes) that increase in frequency, rate, or intensity after presentation of a noxious and putatively painful stimulus. In assays of pain-stimulated behavior, antinociception is manifested by reduced expression of the target behavior; however, the drug effects in assays of pain-stimulated behavior are often not predictive of clinical analgesia in humans (Negus et al., 2006; Whiteside et al., 2008; Mogil, 2009). In particular, drug-induced decreases in the expression of pain-stimulated behaviors can reflect the impaired ability to emit the motor response rather than reduce sensitivity to the noxious stimulus. In contrast, assays of pain-depressed behavior measure behaviors that decrease in frequency, rate, or intensity after presentation of a pain stimulus (e.g., pain-related decreases in feeding or rates of positively reinforced operant behavior). Pain-depressed behaviors play a key role in pain diagnosis in human and veterinary medicine (Cleeland and Ryan, 1994), and the incorporation of procedures that measure pain-depressed behaviors may improve the translational validity in tests of candidate analgesics (Negus et al., 2006, 2010a).

The effects of nAChR agonists have not been examined in assays of pain-depressed behavior. Accordingly, this study compared the effects of nicotine and selective $\alpha 4/6\beta 2$ and $\alpha 7$ nAChR agonists in assays of acute pain-stimulated and pain-depressed behavior that have been used previously to examine the preclinical antinociceptive effects of other drugs, including nonsteroidal anti-inflammatory drugs (Leitl et al., 2014), μ -, δ -, and κ -opioids (Negus, et al., 2010b, 2012; Altarifi et al., 2015), monoamine uptake inhibitors (Rosenberg et al., 2013; Miller et al., 2015), and cannabinoids (Kwilasz and Negus, 2012; Kwilasz et al., 2014). Specifically, an i.p. injection of dilute lactic acid was used as an acute chemical noxious stimulus to stimulate a stretching response and depress the operant responding in an intracranial self-stimulation (ICSS) procedure in rats. The antinociceptive effects of nicotine in both procedures were compared with the effects of the $\alpha 4/6\beta 2$ -selective agonist 5-(123I)iodo-3-[2(S)-2-azetidinylmethoxy]pyridine (5-I-A-85380) (Kulak et al., 2002; Capelli et al., 2011; Mukhin et al., 2000; Liu et al., 2003; Liu, 2013) and the $\alpha 7$ -selective agonist *N*-(3*R*)-1-azabicyclo(2.2.2)oct-3-yl-4-chlorobenzamide (PNU 282987) (Hajós et al., 2005; McLean et al., 2011).

Materials and Methods

Subjects

Male Sprague-Dawley rats (Harlan, Frederick, MD) weighing 310–350 g at the time of surgery were individually housed and maintained on a 12-hour light/dark cycle, with lights on from 6:00 a.m. to 6:00 p.m. Rats had free access to food and water except during testing. Animal maintenance and research were in compliance with National Institutes of Health guidelines on the care and use of animal subjects in research, and all animal use protocols were approved by the Virginia Commonwealth University Institutional Care and Use Committee.

ICSS Behavioral Procedure

Surgery. Rats were anesthetized with isoflurane (2.5–3% in oxygen; Webster Veterinary, Phoenix, AZ) for implantation of stainless steel electrodes. The cathode of each electrode was implanted in the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior and 1.7 mm lateral from the bregma and 8.8 mm below the skull). The anode was wrapped around one of three skull screws to serve as the ground, and the skull screws and electrode assembly were secured with orthodontic resin. Animals were allowed to recover for at least 7 days prior to commencing ICSS training.

Apparatus. Experiments were conducted in sound attenuating chambers that contained modular acrylic test chambers (29.2 × 30.5 × 24.1) equipped with a response lever (4.5 cm wide, extended 2.0 cm through the center of one wall and 3 cm off the floor), stimulus lights (three lights colored red, yellow, and green positioned 7.6 cm directly above the lever), a 2-W white house light, and an ICSS stimulator (Med Associates, St. Albans, VT). Electrodes were connected to the stimulator via bipolar cables and a commutator (Model SL2C; Plastics One, Roanoke, VA). A computer and software program (Med Associates) controlled the stimulator, programming parameters, and data collection.

Training Procedure. Rats were trained under a fixed-ratio 1 schedule of brain stimulation using procedures similar to those described previously for studies with other drugs, including nonsteroidal anti-inflammatory drugs (Leitl et al., 2014), opioids (Negus et al., 2010b, 2012; Altarifi et al., 2015), monoamine uptake inhibitors (Rosenberg et al., 2013; Miller et al., 2015), and cannabinoids (Kwilasz and Negus, 2012; Kwilasz et al., 2014). Each lever press resulted in the delivery of a 0.5-second train of square wave cathodal pulses (0.1-millisecond pulse duration), and stimulation was accompanied by illumination of the stimulus lights above the lever. Responses during the 0.5-second stimulation period did not result in additional stimulation. During the initial phase of training, sessions lasted 30 to 60 minutes, the frequency of stimulation was held constant at 158 Hz, and the stimulation intensity was adjusted to the lowest value that would maintain reinforcement rates of at least 30 stimulations per minute. Frequency manipulations were then introduced during sessions that consisted of sequential 10-minute components. During each component, a descending series of 10 current frequencies (158–56 Hz in 0.05 log increments) was presented, with a 60-second trial at each frequency. A frequency trial began with a 5-second time out followed by a 5-second “priming” phase, during which five noncontingent stimulations were delivered at a rate of one per second. This noncontingent stimulation was followed by a 50-second “response” phase, during which the responding produced an electrical stimulation under a fixed-ratio 1 schedule. Training continued with 3–12 sequential components per day, and the current intensity was adjusted until rats reliably responded during the first three to four frequency trials of all components for at least 3 consecutive days. This intensity (range: 110–250 μ A) was held constant for the remainder of the study.

Testing Procedures. Once training was completed, ICSS testing began. For dose-effect testing with each drug, test sessions consisted of three sequential baseline components followed first by a treatment interval, during which treatments were administered by i.p. injection, and then by three sequential test components. The first component of each session was considered to be a “warm up” component, and data from this component were discarded. Data from the second and third components were used to calculate the baseline parameters of the frequency-rate curves for that session (see *Data Analysis*). During the treatment interval, rats were removed from the ICSS chambers, administered the drug, and placed back into their home cages. After the designated pretreatment time had elapsed, 1.8% lactic acid or its vehicle (sterile water) was administered in a volume of 1 ml/kg, and rats were immediately placed back into their ICSS chambers for the three test components. This 30-minute test period was chosen to match the session length for the stretching studies (see below) and

because our previous studies demonstrated that lactic acid produced a sustained decrease in ICSS for up to 90 minutes (Pereira Do Carmo et al., 2009). The doses and pretreatment times for each test drug were based on preliminary data and previously published behavioral studies in rats (Liu et al., 2003; McLean et al., 2011; Liu, 2013; Freitas, et al., 2015) and were as follows: nicotine (vehicle, 0.01–0.1 mg/kg; 10-minute pretreatment), 5-I-A-85380 (vehicle, 0.01–0.1 mg/kg; 30-minute pretreatment), and PNU 282987 (vehicle, 3.2–32 mg/kg; 30-minute pretreatment). Nicotine and 5-I-A-85380 were each tested in separate groups of six drug-naïve rats; PNU 282987 was tested in a group of four drug-naïve rats and one rat that had been tested previously with nicotine. The dose order was randomized across rats using a Latin-square design. Each week, a rat was tested with a given dose of the test drug in combination with the lactic acid vehicle on one test day and with 1.8% lactic acid on another test day. Test sessions were typically conducted on Tuesdays and Fridays, and three-component training sessions were conducted on Mondays, Wednesdays, and Thursdays.

After completion of the dose-effect studies, additional time-course and mecamylamine antagonism studies were conducted with nicotine. Time-course studies were conducted in a naïve group of six rats. Test sessions consisted of three baseline components followed by a time out, during which 0.1 mg/kg nicotine and 1.8% lactic acid were delivered, and then by two test components. Different test sessions were used to test the different pretreatment time intervals between nicotine and 1.8% lactic acid (30, 100, and 300 minutes), and the order of pretreatment intervals was randomized across rats using a Latin-square design.

Antagonism studies were conducted in five naïve rats and two rats tested previously in nicotine dose-effect studies. Test sessions consisted of three baseline components followed by a 25-minute time out period and then by three test components. Mecamylamine (1.0 mg/kg) or its vehicle was delivered at the beginning of the time out, 15 minutes before nicotine (0.1 mg/kg) or its vehicle, followed 10 minutes later by a 1.8% lactic acid injection immediately before testing. The treatment order was randomized across rats using a Latin-square design.

Data Analysis. The primary dependent variable was the reinforcement rate in stimulations/trial during each frequency trial. To normalize these raw data, reinforcement rates from each trial in each rat were converted to the percent maximum control rate (%MCR). MCR was defined as the mean of the maximal rates observed during the second and third “baseline” components for that day in that rat, and $\%MCR = [(rate\ during\ a\ frequency\ trial)/(MCR)] \times 100$. Normalized ICSS rates at each frequency were averaged across test components within each rat and then across rats to yield a “frequency-rate” curve for each experimental manipulation. Two-way analysis of variance (ANOVA) was used to compare the frequency-rate curves, with ICSS frequency as one variable and dose or time as the second variable. A significant ANOVA was followed by a Holm-Sidak post hoc test, and the criterion for significance was set at $P < 0.05$.

To provide an additional summary measure of ICSS performance, the total number of stimulations per component was calculated as the average of the total stimulations delivered across all 10 frequency trials of each component. Data were expressed as a percentage of the baseline number of stimulations per component. Thus, percent baseline total stimulations was calculated as $(mean\ total\ stimulations\ during\ test\ components \div mean\ total\ stimulations\ during\ baseline\ components) \times 100$. The average data across rats in each experimental condition were compared by a paired *t* test or one-way ANOVA as appropriate. A significant ANOVA was followed by Dunnett's post hoc test. The criterion for significance was set at $P < 0.05$. These data were also used to quantify blockade of acid-induced depression of ICSS as described previously (Rosenberg et al., 2013; Altarifi et al., 2015). Specifically, “percent acid blockade” was quantified using the equation $[(test\ acid)/(baseline\ acid)] \times 100$, where test was the total number of ICSS stimulations after treatment with the drug + acid, acid was the total number of stimulations after acid alone, and baseline was the

total number of stimulations in the absence of the drug or acid. For all drugs producing greater than 50% acid blockade, linear regression was used to calculate ED_{50} and 95% confidence limits, with ED_{50} defined as the effective dose producing 50% acid blockade.

Lactic Acid–Stimulated Stretching Behavioral Procedure

During test sessions, a dose of the test drug was administered i.p. prior to treatment with 1.8% lactic acid (i.p. in a volume of 1.0 ml/kg). Immediately after the acid injection, each rat was placed into an acrylic test chamber (31.0 × 20.1 × 20.0 cm) for a 30-minute observation period. A stretch was operationally defined as a contraction of the abdomen followed by extension of the hind limbs, and the number of stretches during the observation period was counted. The following dose ranges were tested for each drug: nicotine (vehicle, 0.032–1.0 mg/kg), 5-I-A-85380 (vehicle, 0.32–3.2 mg/kg), and PNU 282987 (vehicle, 3.2–32 mg/kg). Each drug was tested using the same pretreatment time as in the ICSS dose-effect studies, and each drug was tested in a different group of six to eight rats. The dose order was randomized using a Latin-square design, and testing was conducted once per week.

After completion of the dose-effect studies, additional time-course and antagonism studies were conducted in the nicotine group. For the time course studies, test sessions consisted of an injection of nicotine (1.0 mg/kg) followed 10, 30, 100, or 300 minutes later by an acid injection immediately before testing. A dose of 1.0 mg/kg nicotine was chosen for the time-course studies because it produced the greatest antinociceptive effect. Pretreatment times were randomized across rats using a Latin-square design, and test sessions were separated by 1 week. For antagonism studies, 1.0 mg/kg mecamylamine or its vehicle was delivered 15 minutes before 1.0 mg/kg nicotine or its vehicle, and acid was delivered 10 minutes after nicotine. The treatment order was randomized across rats using a Latin-square design, and test sessions were separated by 1 week.

Data Analysis. The primary dependent variable was the number of stretches counted during each observation period in each rat. Data were averaged across rats and evaluated by one-way ANOVA. A significant ANOVA was followed by Tukey's post hoc test, and the criterion for significance was set at $P < 0.05$. For drugs producing a greater than 50% reduction in stretching relative to vehicle treatment, linear regression was used to calculate ED_{50} and 95% confidence limits, with ED_{50} defined as the effective dose to reduce stretching to 50% of vehicle control. ED_{50} values were considered to be significantly different if 95% confidence limits did not overlap.

Drugs

Lactic acid, (–)-nicotine hydrogen tartrate, and mecamylamine HCl were purchased from Sigma-Aldrich (St. Louis, MO). 5-I-A-85380 and PNU 282987 were synthesized at the Research Triangle Institute and generously provided by Dr. Ivy Carroll. Lactic acid was prepared in sterile water. Nicotine, mecamylamine, 5-I-A-85380 2HCl, and PNU 282987 HCl were prepared in sterile saline. Nicotine doses are expressed as the free base of the drug, whereas the mecamylamine, 5-I-A-85380, and PNU 282987 doses are expressed as the salt forms. All solutions were injected intraperitoneally in a volume of 1 ml/kg.

Results

Effects of Nicotine, 5-I-A-85380, and PNU 282987 on Acid-Stimulated Stretching. Across all 20 rats used for studies of acid-stimulated stretching, i.p. administration of 1.8% lactic acid (1.0 ml/kg) after drug vehicle pretreatment elicited a mean $\pm$ S.E.M. of 14.75 ± 3.0 stretches. Figure 1 shows that nicotine produced dose-dependent, time-dependent, and mecamylamine-reversible antinociception in the assay of acid-stimulated stretching. Figure 1A shows that stretching

Fig. 1. Nicotine effects on acid-stimulated stretching. (A) Effects of nicotine (0.032–1.0 mg/kg) or its vehicle (Veh) administered 10 minutes before acid. Abscissa, dose of nicotine in milligrams per kilogram. The ordinate for all panels is the number of stretches observed during a 30-minute observation period. (B) Effects of nicotine (1.0 mg/kg) administered 10–300 minutes before acid. The effects of vehicle administered 10 minutes before acid are included for comparison. The abscissa is the time after nicotine administration in minutes. (C) Effects of mecamylamine (Mec) (1.0 mg/kg) or its vehicle on the antinociceptive effects of nicotine (Nic) (1.0 mg/kg, 10-minute pretreatment). The effects of mecamylamine vehicle + nicotine vehicle before acid are included for comparison. The abscissa is the dose of Nic and mecamylamine in milligrams per kilogram. One-way ANOVA indicated the significant main effects of the nicotine dose in (A) ($F_{4,20} = 26.97$; $P < 0.0001$), time in (B) ($F_{4,20} = 28.84$; $P < 0.0001$), and mecamylamine dose in (C) ($F_{2,10} = 48.64$; $P < 0.0001$). * $P < 0.05$ compared with Veh (A and B) or Veh+Veh (C) and $^{\$}P < 0.05$ compared with 1.0 Nic + Veh (C) as determined by Dunnett post hoc test in (A and B) or Tukey post hoc test (C). All bars show the mean $\pm$ S.E.M. in six rats.

was significantly lower 10 minutes after administration of 0.1, 0.32, and 1.0 mg/kg nicotine than after nicotine vehicle, and the nicotine ED_{50} value is reported in Table 1.

Figure 1B shows that 1.0 mg/kg nicotine produced a significant reduction in acid-stimulated stretching from 10 to 100 minutes after its administration. Figure 1C shows that 1.0 mg/kg of mecamylamine significantly blocked the antinociceptive effect of 1.0 mg/kg nicotine.

Figure 2 shows that the $\alpha 4/6\beta 2$ -selective agonist 5-I-A-85380, but not the $\alpha 7$ -selective agonist PNU 282987, also produced a dose-dependent decrease in acid-stimulated stretching. Figure 2A shows that stretching was significantly lower 30 minutes after administration of 1.0 and 3.2 mg/kg 5-I-A-85380 than after the 5-I-A-85380 vehicle, and the ED_{50} value is shown in Table 1. 5-I-A-85380 was significantly less potent than nicotine. Conversely, Fig. 2B shows no significant reduction in stretching 30 minutes after administration of PNU 282987 doses of up to 32 mg/kg.

Effects of Nicotine, 5-I-A-85380, and PNU 282987 on Acid-Induced Depression of ICSS. For the 27 rats used in these studies, the average $\pm$ S.E.M. baseline MCR was 57.02 ± 2.69 stimulations per trial, and the average $\pm$ S.E.M. number of total baseline stimulations per component was 258.55 ± 24.07 . Figure 3 shows the effects of treatment with the acid vehicle or 1.8% lactic acid on ICSS for all 27 rats. After vehicle treatment, the maximum reinforcement rates were usually observed at the highest stimulation frequencies (112–158 Hz), and the response decreased in a frequency-dependent

manner. Administration of 1.8% lactic acid depressed ICSS, producing a rightward shift in the frequency-rate curve (Fig. 3A) and a decrease in the total number of stimulations per component (Fig. 3B). This acid-induced depression of ICSS provided a measure of pain-related behavioral depression, and nicotinic compounds were evaluated for their ability to block this acid-induced depression of ICSS.

Figure 4 shows that 10-minute pretreatment with nicotine produced a dose-dependent and complete blockade of acid-induced depression of ICSS and also facilitated control ICSS in the absence of the noxious acid stimulus. When administered as a pretreatment to the acid vehicle, 0.01 mg/kg nicotine had no effect on ICSS, but higher nicotine doses of 0.032 and 0.1 mg/kg nicotine produced leftward shifts in the ICSS frequency-rate curve and significant facilitation of ICSS at intermediate frequencies of brain stimulation (79–100 Hz) (Fig. 4A). When administered as a pretreatment to 1.8% lactic acid, all nicotine doses from 0.01 to 0.1 mg/kg significantly ameliorated acid-induced depression of ICSS across a broad range of frequencies ranging from 79 to 141 Hz (Fig. 4B). Thus, nicotine was slightly more potent to produce an antinociceptive blockade of acid-induced depression of ICSS than to facilitate control ICSS. Summary data are shown in Fig. 4C, and the nicotine ED_{50} value is reported in Table 1. Nicotine was significantly more potent in blocking acid-induced depression of ICSS than in blocking acid-stimulated stretching. Nicotine doses higher than 0.1 mg/kg were tested in pilot studies in some rats; however, these doses decreased rates of ICSS, and systematic studies with higher doses were not pursued.

Figure 5 shows that nicotine produced a time-dependent and mecamylamine-reversible antinociception in the procedure of acid-induced depression of ICSS. A dose of 0.1 mg/kg nicotine blocked acid-induced depression of ICSS after 10 minutes (Fig. 4), and Fig. 5A shows that 0.1 mg/kg nicotine also significantly attenuated acid-induced depression of ICSS after 30 minutes but not after later pretreatment times. Figure 5B shows that the effects of 0.1 mg/kg nicotine were blocked by a dose of 1.0 mg/kg mecamylamine, which did not alter acid-induced depression of ICSS when it was given without nicotine.

TABLE 1

ED_{50} values in milligram per kilogram (95% confidence limits) for nicotinic drugs to produce antinociception in the assays of acid-stimulated stretching or acid-induced depression of ICSS

	Acid-Stimulated Stretching	Acid-Depressed ICSS
Nicotine	0.14 (0.09–0.20)	0.011 (0.006–0.023) [†]
5-I-A-85380	1.19 (0.48–2.97)*	0.012 (0.006–0.021) [†]
PNU 282987	Inactive	Inactive

*Significantly different from nicotine ED_{50} within a given assay.

[†]Significantly different from the ED_{50} for that drug in the assay of acid-stimulated stretching.

Fig. 2. Effects of 5-I-A-85380 and PNU 282987 on acid-stimulated stretching. The abscissae are the dose of 5-I-A-85380 (A) or PNU 282987 (B) in milligrams per kilogram administered 30 minutes before acid. The ordinates are the number of stretches observed during a 30-minute observation period. One-way ANOVA indicated the significant effect of 5-I-A-85380 treatment in (A) ($F_{3,21} = 14.12$; $P < 0.0001$) and no significant effect of PNU 282987 treatment in (B) ($F_{3,15} = 2.14$; $P = 0.14$). * $P < 0.05$ compared with vehicle (Veh) as determined by Dunnett's post hoc test. All bars show the mean $\pm$ S.E.M. in eight rats (5-I-A-85380) or six rats (PNU 282987).

Figure 6 shows the effects of 5-I-A-85380 and PNU 282987 on ICSS in the absence or presence of the acid noxious stimulus. Like nicotine, 5-I-A-85380 (0.01–0.1 mg/kg) produced a dose-dependent and complete blockade of acid-induced depression of ICSS, and it was slightly more potent in blocking acid-induced depression of ICSS than in facilitating control ICSS in the absence of the acid noxious stimulus (Fig. 6, A–C). The ED_{50} value for 5-I-A-85380 is shown in Table 1. There was no difference in the potencies of nicotine and 5-I-A-85380 to block acid-induced depression of ICSS, and like nicotine, 5-I-A-85380 was significantly more potent in blocking acid-induced depression of ICSS than in acid-stimulated stretching. 5-I-A-85380 doses higher than 0.1 mg/kg were also tested in pilot studies in some rats; however, the high dose of 3.2 mg/kg caused death in some rats, and as a result, further studies with higher doses were not pursued. In contrast to nicotine and 5-I-A-85380, PNU 282987 (3.2–32 mg/kg) failed to alter control ICSS in the absence of the noxious stimulus and also failed to alleviate acid-induced depression of ICSS (Fig. 6, D–F).

Discussion

This study compared the effects of nicotine with the effects of selective $\alpha 4/6\beta 2$ and $\alpha 7$ nAChR agonists in preclinical assays of pain-stimulated and pain-depressed behavior in rats. There were three main findings. First, both nicotine and the more selective $\alpha 4/6\beta 2$ agonist 5-I-A-85380 produced antinociception in both assays, whereas the $\alpha 7$ agonist PNU 282987 did not. Second, both nicotine and 5-I-A-85380 were >10-fold more potent to produce antinociception in the assay of acid-depressed ICSS than in the assay of acid-stimulated stretching. Lastly, both nicotine and 5-I-A-85380 were also more potent to alleviate acid-induced depression of ICSS than to facilitate ICSS in the absence of the noxious stimulus. Taken together, these results suggest that $\alpha 4\beta 2$ nAChR agonists may be especially effective to treat signs of pain-related behavioral depression; however, as discussed further below, nonselective behavioral effects of these compounds may contribute to apparent antinociception.

Fig. 3. Acid-induced depression of ICSS. (A) ICSS frequency-rate curves determined after pretreatment with drug vehicle (Veh) + lactic acid (LA) Veh or Veh + 1.8% LA for all rats in this study. The abscissa is the frequency of electrical brain stimulation in Hertz (log scale). The ordinate is the rate of ICSS expressed as %MCR. Two-way ANOVA indicated the significant main effects of frequency ($F_{9,234} = 195.4$; $P < 0.0001$) and acid treatment ($F_{1,26} = 105.2$; $P < 0.0001$) and a significant interaction ($F_{9,234} = 15.23$; $P < 0.0001$). Filled symbols indicate a significant difference from Veh + LA Veh as determined by the Holm-Sidak post hoc test; $P < 0.05$. (B) Summary data for the 1.8% lactic acid effects on the total number of stimulations per component. The abscissa is the pretreatment condition. The ordinate is the percent baseline number of stimulations per component. Veh + 1.8% lactic acid significantly depressed ICSS compared with Veh + LA Veh as determined by paired t test ($t_{26} = 9.14$; $*P < 0.0001$). All data show the mean $\pm$ S.E.M. in 27 rats.

Fig. 4. Effects of nicotine on control and acid-depressed ICSS. ICSS frequency-rate curves determined when nicotine (0.01–0.1 mg/kg) was administered as a pretreatment to lactic acid (LA) vehicle (Veh) (A) or 1.8% LA (B). The abscissae are the frequency of electrical brain stimulation in Hertz (log scale). The ordinates are %MCR. Filled symbols indicate a significant difference from nicotine Veh + LA Veh in (A) or nicotine Veh + 1.8% LA in (B) as determined by Holm-Sidak post hoc test; $P < 0.05$. All points show the mean data from six rats, and error bars are omitted for clarity. Two-way ANOVA results were as follows: (A) significant main effects of frequency ($F_{9,45} = 117.5$; $P < 0.0001$) and nicotine dose ($F_{3,15} = 7.67$; $P = 0.002$) but not a significant interaction ($F_{27,135} = 1.54$; $P = 0.06$); and (B) significant main effects of frequency ($F_{9,45} = 44.16$; $P < 0.0001$) and nicotine dose ($F_{3,15} = 7.83$; $P = 0.002$) and a significant interaction ($F_{27,135} = 1.71$; $P = 0.02$). (C) Summary data for the nicotine effects on the total number of stimulations per component when nicotine was administered as a pretreatment to the acid vehicle (open bars) or 1.8% lactic acid (filled bars). The abscissae are the dose of nicotine in milligrams per kilogram. The ordinate is the percent baseline number of stimulations per component. * $P < 0.05$ compared with Nic Veh + LA Veh as determined by paired t test. Upward arrows indicate that nicotine produced a significant increase in ICSS at one or more frequencies in the analysis of the full frequency-rate curves in (A and B). All bars show the mean $\pm$ S.E.M. in six rats.

nAChR Agonist Effects on Acid-Stimulated Stretching. The potency, time course, and mecamylamine antagonism of nicotine antinociception in the assay of acid-stimulated stretching is consistent with previous studies of nicotine in preclinical assays of pain-stimulated behavior. For example, previous studies in mice found that nicotine dose-dependently decreased stretching elicited by intraperitoneal acid administration (Han et al., 2005; Kwon et al., 2008). Nicotine also

blocked other pain-stimulated behaviors, such as tail- and paw-withdrawal responses from noxious thermal stimuli (Tripathi et al., 1982; Aceto et al., 1983; Rogers and Iwamoto, 1993) and withdrawal responses in subjects rendered hypersensitive to thermal or mechanical stimuli by inflammatory or neuropathic manipulations (Damaj et al., 1999; Abidin et al., 2006; Saika et al., 2015). As in the present study, nicotine antinociception is often shown to be dose and/or time dependent and sensitive to

Fig. 5. Time course and mecamylamine (Mec) antagonism of 0.1 mg/kg nicotine (Nic) antinociception in the assay of acid-depressed ICSS. (A) The abscissa is the pretreatment interval between the administration of 0.1 mg/kg nicotine and 1.8% lactic acid. Effects of nicotine vehicle + lactic acid vehicle (Veh + Veh) and nicotine vehicle + 1.8% lactic acid (Veh + 1.8% LA) are also shown for comparison, with the nicotine vehicle administered 10 minutes before the acid vehicle or acid. The ordinate is the percentage of baseline total number of stimulations per component. * $P < 0.02$ compared with Veh + Veh as determined by paired t test ($t_5 = 3.33$). One-way ANOVA of data in the filled bars indicated a significant effect of time ($F_{3,15} = 3.90$; $P = 0.03$). * $P < 0.05$ compared with Veh + 1.8% LA as determined by Dunnett's post hoc test. All bars show the mean $\pm$ S.E.M. in six rats. (B) Effects of 15-minute pretreatment with Mec (1.0 mg/kg) or its vehicle and 10-minute pretreatment with Nic (0.1 mg/kg) or its vehicle before acid treatment. Effects of mecamylamine vehicle + nicotine vehicle + acid vehicle are included for comparison. The abscissa is treatment with 1.0 mg/kg Mec, 0.1 mg/kg Nic, 1.8% LA, or their respective vehicles. The ordinate is the percentage of baseline total number of stimulations per component. * $P = 0.006$ compared with Veh + Veh + Veh as determined by paired t test ($t_6 = 4.17$). One-way ANOVA of data in the filled bars indicated the significant main effect of treatment ($F_{3,18} = 14.18$; $P < 0.0001$). * $P < 0.05$ compared with Veh + Veh + 1.8% LA as determined by Dunnett's post hoc test. All bars show the mean $\pm$ S.E.M. in seven rats.

Fig. 6. Effects of 5-I-A-85380 (A–C) and PNU 282987 (D–F) on control and acid-depressed ICSS. Left and center panels show the ICSS frequency-rate curves that were determined when 5-I-A-85380 (0.01–0.1 mg/kg) or PNU 282987 (3.2–32 mg/kg) was administered as a pretreatment to lactic acid (LA) vehicle (Veh) (A and D) or 1.8% LA (B and E). The abscissae are the frequency of electrical brain stimulation in Hertz (log scale). The ordinates are % MCR. Filled symbols indicate a significant difference from Veh + LA Veh in (A) or Veh + 1.8% LA in (B) as determined by the Holm-Sidak post hoc test; $P < 0.05$. All points show the mean data in six rats (5-I-A-85380) or five rats (PNU 282987), and error bars are omitted for clarity. Two-way ANOVA results for each panel were as follows: (A) significant main effects of frequency ($F_{9,45} = 29.84$; $P < 0.0001$) and 5-I-A-85380 dose ($F_{3,15} = 8.88$; $P = 0.001$) and a significant interaction ($F_{27,135} = 3.63$; $P < 0.0001$); (B) significant main effects of frequency ($F_{9,45} = 76.18$; $P < 0.0001$) and 5-I-A-85380 dose ($F_{3,15} = 6.17$; $P = 0.006$) and a significant interaction ($F_{27,135} = 2.52$; $P = 0.0003$); (D) significant main effect of frequency ($F_{9,36} = 150.5$; $P < 0.0001$) but not PNU 282987 dose ($F_{3,12} = 0.88$; $P = 0.47$) and no significant interaction ($F_{27,108} = 0.78$; $P = 0.77$); (E) significant main effect of frequency ($F_{9,36} = 51.75$; $P < 0.0001$) but not PNU 282987 dose ($F_{3,12} = 0.68$; $P = 0.58$) and no significant interaction ($F_{27,108} = 0.49$; $P = 0.98$). Summary data for the effects of 5-I-A-85380 (C) and PNU 282987 (F) on the total number of stimulations per component when drugs were administered as a pretreatment to the acid vehicle (open bars) or 1.8% lactic acid (filled bars). The abscissae are the drug dose in milligrams per kilogram. The ordinates are the percent baseline number of stimulations per component. * $P < 0.05$ compared with Veh + LA Veh as determined by paired t test. Upward arrows indicate that 5-I-A-85380 produced a significant increase in ICSS at one or more frequencies in the analysis of the full frequency-rate curves in (A and B). All bars show the mean $\pm$ S.E.M. in six rats (5-I-A-85380) or five rats (PNU 282987).

mecamylamine antagonism (Sahley and Berntson, 1979; AlSharari et al., 2012). The effectiveness of 5-I-A-85380 to block acid-stimulated stretching is also consistent with previous reports of antinociception by A-85380 and other $\alpha 4/6\beta 2$ agonists in assays of pain-stimulated behavior. 5-I-A-85380 was created by the introduction of an iodine substituent onto the pyridine ring of A-85380 to generate an iodinated compound suitable for imaging studies (Mukhin et al., 2000; Rueter et al., 2006), and the parent compound A-85380 has a broad-spectrum antinociception profile with efficacy in acute, inflammatory, and neuropathic pain models that rely on pain-stimulated behaviors (Curzon et al., 1998; Rueter et al., 2003).

Likewise, other agonists with selectivity for $\alpha 4/6\beta 2$ receptors, such as NS3956 (Rode et al., 2012) and A-366833 (Ji et al., 2007; Nirogi et al., 2011), also produced antinociception in a broad range of pain-stimulated behavior assays in mice and rats.

Some evidence has accumulated to suggest that activation of $\alpha 7$ nAChRs may also be sufficient to produce antinociception in rodents. For example, intrathecal or intracerebroventricular

administration of the $\alpha 7$ agonist choline produced MLA-reversible antinociception in a tail-flick assay in mice, and intravenous choline produced MLA-reversible antinociception in a formalin test in mice (Damaj et al., 2000; Wang et al., 2005). However, other studies failed to observe antinociception in rodents after treatment with drugs characterized as $\alpha 7$ agonists. For example, intravenous choline was not effective in a hot-plate assay in mice, and although the other $\alpha 7$ agonist SSR-180711 reduced formalin-induced licking and flinching in rats, the effect was attributed to a nonspecific reduction of movement (Wang et al., 2005; Gao et al., 2010). In the present study, PNU 282987 failed to produce antinociception at doses of up to 32 mg/kg. This lack of effectiveness is probably not due to inadequate dosing because PNU 282987 was tested up to doses that did reverse MK-801-induced deficits on measures of cognitive performance in rats (Jones et al., 2014). Overall, the present results agree with previous studies that failed to observe antinociception in assays of pain-stimulated behavior with $\alpha 7$ agonists in rats.

nAChR Agonist Effects on Acid-Depressed ICSS. This is the first study to examine the effects of nAChR agonists in an assay of pain-depressed behavior, and the drug effects in the assay of acid-depressed ICSS were qualitatively similar to the effects in the assay of acid-stimulated stretching. Thus, nicotine produced dose-dependent, time-dependent, and mecamylamine-reversible blockade of acid-induced depression of ICSS, and 5-I-A-85380 also produced dose-dependent antinociception, whereas PNU 282987 did not. The effects of nicotine and 5-I-A-85380 are also qualitatively similar to the effects in this procedure produced by clinically effective analgesics, including the NSAID ketoprofen and mu-opioid receptor agonists like morphine (Pereira Do Carmo et al., 2009; Leidl et al., 2014; Altarifi et al., 2015). Moreover, the effects of nicotine and 5-I-A-85380 differed from the effects of some other drugs, including kappa-opioid receptor agonists (Negus, et al., 2010b, 2012; Leidl et al., 2014) and cannabinoid receptor agonists (Kwilasz and Negus, 2012; Kwilasz et al., 2014), which produce antinociception in many assays of pain-stimulated behavior but fail to produce antinociception in assays of pain-depressed behavior. Taken together, the effectiveness of nicotine and 5-I-A-85380 to block both pain-stimulated and pain-depressed behaviors in rats supports further consideration of these and related compounds as candidate analgesics. Conversely, the failure of PNU 282987 to produce antinociception in either procedure does not support further consideration of $\alpha 7$ agonists.

Although the effects of nicotine and 5-I-A-85380 were qualitatively similar in assays of acid-stimulated stretching and acid-depressed ICSS, both compounds were much more potent in blocking acid-induced depression of ICSS. Specifically, nicotine was 10 times more potent and 5-I-A-85380 was 100 times more potent in blocking acid-induced depression of ICSS than in blocking acid-stimulated stretching. This differs from the effects of morphine and most other mu-opioid analgesics, which display similar potencies in these two assays (Pereira Do Carmo et al., 2009; Altarifi et al., 2015). In the present study, the low doses of nicotine and 5-I-A-85380 that blocked acid-induced depression of ICSS have also been shown to increase other behaviors, whereas the high doses of these compounds that reduced acid-stimulated stretching have also been shown to depress other behaviors. For example, nicotine at doses of up to 0.1–0.32 mg/kg produced dose-dependent increases in spontaneous locomotor activity, rates of food-maintained operant responding, and rates of ICSS, whereas doses $\geq$ 0.1–0.32 mg/kg decreased rates of all these behaviors (Clarke and Kumar, 1983; Goldberg et al., 1989; Cohen et al., 1991; Huston-Lyons and Kornetsky, 1992; Bauco and Wise, 1994; Whiteaker et al., 1995; Spiller et al., 2009; Freitas et al., 2015). The effects of 5-I-A-85380 have not been examined extensively in these other procedures in rats, although we recently reported that 5-I-A-85380 doses of up to 1.0 mg/kg produced mecamylamine- and dihydro- β -erythroidine-reversible facilitation of ICSS in the absence of a noxious stimulus (Freitas et al., 2015). Moreover, like nicotine in the present study, 5-I-A-85380 was only slightly more potent in blocking acid-induced depression of ICSS than in increasing control ICSS rates in the absence of the acid noxious stimulus, and 5-I-A-85380 decreased acid-stimulated stretching only at high doses $\geq$ 1.0 mg/kg, which also produced audible and labored breathing (1.0 mg/kg) or lethality in some animals (3.2 mg/kg).

Taken together, these results suggest that nonselective behavioral activation may have contributed to apparent nicotine and 5-I-A-85380 antinociception in the assay of acid-depressed ICSS, and nonselective behavioral impairment may have contributed to apparent antinociception by these compounds in the assay of acid-stimulated stretching. This evidence for nonselective behavioral stimulation/impairment in apparent antinociception by nicotinic agonists may also be consistent with the narrow therapeutic window and emergence of undesirable effects at analgesic doses for nAChR agonists in studies of acute pain in humans (Greiff et al., 1993; Weingarten et al., 2008; Mishriky and Habib, 2014).

nAChR Agonist Effects on ICSS in the Absence of Acid. This study focused primarily on the effectiveness of nAChR agonists to block acid-induced depression of ICSS; however, all the drugs were also tested for their effects on ICSS in the absence of noxious stimulation and the results are consistent with previous studies of nAChR agonist effects on ICSS. For example, previous studies have also shown that ICSS was facilitated after treatment with low but not high nicotine doses or with 5-I-A-85380 or the other $\alpha 4/6\beta 2$ agonist SIB-1765F, whereas $\alpha 7$ agonists, such as ARR-17779, did not alter ICSS (Bauco and Wise, 1994; Panagis et al., 2000; Spiller et al., 2009; Freitas et al., 2015). Insofar as drug-induced facilitation of ICSS is often interpreted as evidence of abuse liability (Negus and Miller, 2014), the present results are consistent with the conclusion that abuse liability of $\alpha 4/6\beta 2$ agonists may be one factor that limits their utility as candidate analgesics.

Role for $\alpha 6^*$ Receptors. 5-I-A-85380 is often described as an $\alpha 4\beta 2$ -selective agonist (Mukhin et al., 2000; Sihver et al., 2000; Liu et al., 2003; Liu, 2013), and the results of this study support previous evidence to implicate $\alpha 4\beta 2$ receptors in antinociception by nAChR agonists. However, 5-I-A-85380 also binds to $\alpha 6^*$ receptors, which are nAChRs that contain the $\alpha 6$ subunit instead of or in addition to $\alpha 4$ subunits (Kulak et al., 2002; Capelli et al., 2011). Moreover, $\alpha 6^*$ nAChRs are located in both primary sensory neurons and components of the mesolimbic dopamine system, and activation of these receptors is associated with both antinociception in assays of pain-stimulated behavior and neurochemical and behavioral evidence for stimulation of the mesolimbic dopamine system (Brunzell, 2012; Wieskopf et al., 2015). In view of these considerations, the present results do not exclude a role for $\alpha 6^*$ nAChRs in mediating the effects of nAChR agonists on pain-depressed ICSS in rats.

Authorship Contributions

Participated in research design: Freitas, Carroll, Negus.

Conducted experiments: Freitas.

Performed data analysis: Freitas, Negus.

Wrote or contributed to the writing of the manuscript: Freitas, Carroll, Negus.

References

- Abdin MJ, Morioka N, Morita K, Kitayama T, Kitayama S, Nakashima T, and Dohi T (2006) Analgesic action of nicotine on tibial nerve transection (TNT)-induced mechanical allodynia through enhancement of the glycinergic inhibitory system in spinal cord. *Life Sci* 80:9–16.
- Aceto MD, Awaya H, Martin BR, and May EL (1983) Antinociceptive action of nicotine and its methiodide derivatives in mice and rats. *Br J Pharmacol* 79:869–876.
- AlSharari SD, Carroll FI, McIntosh JM, and Damaj MI (2012) The antinociceptive effects of nicotinic partial agonists varenicline and sazetidine-A in murine acute and tonic pain models. *J Pharmacol Exp Ther* 342:742–749.
- Altarifi AA, Rice KC, and Negus SS (2015) Effects of μ -opioid receptor agonists in assays of acute pain-stimulated and pain-depressed behavior in male rats: role of

- μ -agonist efficacy and noxious stimulus intensity. *J Pharmacol Exp Ther* **352**: 208–217.
- Bauco P and Wise RA (1994) Potentiation of lateral hypothalamic and midline mesencephalic brain stimulation reinforcement by nicotine: examination of repeated treatment. *J Pharmacol Exp Ther* **271**:294–301.
- Brunzell DH (2012) Preclinical evidence that activation of mesolimbic alpha 6 subunit containing nicotinic acetylcholine receptors supports nicotine addiction phenotype. *Nicotine Tob Res Off J Soc Res Nicotine Tob* **14**:1258–1269.
- Capelli AM, Castelletti L, Chen YH, Van der Keyl H, Pucci L, Oliosi B, Salvagno C, Bertani B, Gotti C, and Powell A, et al. (2011) Stable expression and functional characterization of a human nicotinic acetylcholine receptor with $\alpha 6\beta 2$ properties: discovery of selective antagonists. *Br J Pharmacol* **163**:313–329.
- Clarke PB and Kumar R (1983) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol* **78**:329–337.
- Cleeland CS and Ryan KM (1994) Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore* **23**:129–138.
- Cohen C, Welzl H, and Bättig K (1991) Effects of nicotine, caffeine, and their combination on locomotor activity in rats. *Pharmacol Biochem Behav* **40**:121–123.
- Cooley JE, Villarosa GA, Lombardo TW, Moss RA, Fowler SC, and Sult S (1990) Effect of pCPA on nicotine-induced analgesia. *Pharmacol Biochem Behav* **36**:413–415.
- Curzon P, Nikkel AL, Bannon AW, Arneric SP, and Decker MW (1998) Differences between the antinociceptive effects of the cholinergic channel activators A-85380 and (+/-)-epibatidine in rats. *J Pharmacol Exp Ther* **287**:847–853.
- Damaj MI, Fei-Yin M, Dukat M, Glascow W, Glennon RA, and Martin BR (1998) Antinociceptive responses to nicotinic acetylcholine receptor ligands after systemic and intrathecal administration in mice. *J Pharmacol Exp Ther* **284**:1058–1065.
- Damaj MI, Glascow W, Aceto MD, and Martin BR (1999) Antinociceptive and pharmacological effects of metanictine, a selective nicotinic agonist. *J Pharmacol Exp Ther* **291**:390–398.
- Damaj MI, Meyer EM, and Martin BR (2000) The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. *Neuropharmacology* **39**:2785–2791.
- Damaj MI, Welch SP, and Martin BR (1994) Nicotine-induced antinociception in mice: role of G-proteins and adenylate cyclase. *Pharmacol Biochem Behav* **48**:37–42.
- Damaj MI, Welch SP, and Martin BR (1995) In vivo pharmacological effects of dihydro-beta-erythroidine, a nicotinic antagonist, in mice. *Psychopharmacology (Berl)* **117**:67–73.
- Ditre JW, Brandon TH, Zale EL, and Meagher MM (2011) Pain, nicotine, and smoking: research findings and mechanistic considerations. *Psychol Bull* **137**: 1065–1093.
- Donnelly-Roberts DL, Puttfarcken PS, Kuntzweiler TA, Briggs CA, Anderson DJ, Campbell JE, Piattoni-Kaplan M, McKenna DG, Wasicak JT, and Holladay MW, et al. (1998) ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine]: a novel, orally effective analgesic acting via neuronal nicotinic acetylcholine receptors: I. In vitro characterization. *J Pharmacol Exp Ther* **285**:777–786.
- Dukat M, Wesolowska A, Alley G, Young S, Abdurakhmanova GR, Navarro HA, Young R, and Glennon RA (2010) MD-354 selectively antagonizes the antinociceptive effects of (-)-nicotine in the mouse tail-flick assay. *Psychopharmacology (Berl)* **210**: 547–557.
- Eaton JB, Peng J-H, Schroeder KM, George AA, Fryer JD, Krishnan C, Buhlman L, Kuo Y-P, Steinlein O, and Lukas RJ (2003) Characterization of human alpha 4 beta 2 nicotinic acetylcholine receptors stably and heterologously expressed in native nicotinic receptor-null SH-EP1 human epithelial cells. *Mol Pharmacol* **64**:1283–1294.
- Flores CM (2000) The promise and pitfalls of a nicotinic cholinergic approach to pain management. *Pain* **88**:1–6.
- Freitas K, Carroll FI, and Damaj MI (2013a) The antinociceptive effects of nicotinic receptors $\alpha 7$ -positive allosteric modulators in murine acute and tonic pain models. *J Pharmacol Exp Ther* **344**:264–275.
- Freitas K, Carroll FI, and Negus SS (2015) Comparison of effects produced by nicotine and the $\alpha 4\beta 2$ -selective agonist 5-I-A-85380 on intracranial self-stimulation in rats. *Exp Clin Psychopharmacol*, in press.
- Freitas K, Ghosh S, Ivy Carroll F, Lichtman AH, and Imad Damaj M (2013b) Effects of $\alpha 7$ positive allosteric modulators in murine inflammatory and chronic neuropathic pain models. *Neuropharmacology* **65**:156–164.
- Gao M, Jin Y, Yang K, Zhang D, Lukas RJ, and Wu J (2010) Mechanisms involved in systemic nicotine-induced glutamatergic synaptic plasticity on dopamine neurons in the ventral tegmental area. *J Neurosci Off J Soc Neurosci* **30**:13814–13825.
- Gerzanich V, Peng X, Wang F, Wells G, Anand R, Fletcher S, and Lindstrom J (1995) Comparative pharmacology of epibatidine: a potent agonist for neuronal nicotinic acetylcholine receptors. *Mol Pharmacol* **48**:774–782.
- Goldberg SR, Risner ME, Stolerman IP, Reavill C, and Garcha HS (1989) Nicotine and some related compounds: effects on schedule-controlled behaviour and discriminative properties in rats. *Psychopharmacology (Berl)* **97**:295–302.
- Greiff L, Wollmer P, Erjefält I, Andersson M, Pipkorn U, and Persson CG (1993) Effects of nicotine on the human nasal mucosa. *Thorax* **48**:651–655.
- Hajós M, Hurst RS, Hoffmann WE, Krause M, Wall TM, Higdon NR, and Groppi VE (2005) The selective alpha7 nicotinic acetylcholine receptor agonist PNU-282987 [N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats. *J Pharmacol Exp Ther* **312**:1213–1222.
- Han K-J, Choi S-S, Lee J-Y, Lee H-K, Shim E-J, Kwon MS, Seo Y-J, and Suh H-W (2005) Antinociceptive effect of nicotine in various pain models in the mouse. *Arch Pharm Res* **28**:209–215.
- Huston-Lyons D and Kornetsky C (1992) Effects of nicotine on the threshold for rewarding brain stimulation in rats. *Pharmacol Biochem Behav* **41**:755–759.
- Iwamoto ET (1991) Characterization of the antinociception induced by nicotine in the pedunculo-pontine tegmental nucleus and the nucleus raphe magnus. *J Pharmacol Exp Ther* **257**:120–133.
- Jensen AA, Mikkelsen I, Frølund B, Bräuner-Osborne H, Falch E, and Krogsgaard-Larsen P (2003) Carbamoylcholine homologs: novel and potent agonists at neuronal nicotinic acetylcholine receptors. *Mol Pharmacol* **64**:865–875.
- Ji J, Bunnelle WH, Anderson DJ, Faltynek C, Dyhring T, Ahning PK, Rueter LE, Curzon P, Buckley MJ, and Marsh KC, et al. (2007) A-366833: a novel nicotinonitrile-substituted 3,6-diazabicyclo[3.2.0]-heptane alpha4beta2 nicotinic acetylcholine receptor selective agonist: synthesis, analgesic efficacy and tolerability profile in animal models. *Biochem Pharmacol* **74**:1253–1262.
- Jones KM, McDonald IM, Bourin C, Olson RE, Bristow LJ, and Easton A (2014) Effect of alpha7 nicotinic acetylcholine receptor agonists on attentional set-shifting impairment in rats. *Psychopharmacology (Berl)* **231**:673–683.
- Kem WR, Mahnir VM, Papke RL, and Lingle CJ (1997) Anabaseine is a potent agonist on muscle and neuronal alpha-bungarotoxin-sensitive nicotinic receptors. *J Pharmacol Exp Ther* **283**:979–992.
- Kulak JM, Sum J, Musachio JL, McIntosh JM, and Quik M (2002) 5-Iodo-A-85380 binds to alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors (nAChRs) as well as alpha4beta2* subtypes. *J Neurochem* **81**:403–406.
- Kwilas AJ, Abdullah RA, Poklis JL, Lichtman AH, and Negus SS (2014) Effects of the fatty acid amide hydrolase inhibitor URB597 on pain-stimulated and pain-depressed behavior in rats. *Behav Pharmacol* **25**:119–129.
- Kwilas AJ and Negus SS (2012) Dissociable effects of the cannabinoid receptor agonists $\Delta 9$ -tetrahydrocannabinol and CP55940 on pain-stimulated versus pain-depressed behavior in rats. *J Pharmacol Exp Ther* **343**:389–400.
- Kwon M-S, Seo Y-J, Choi S-M, Lee J-K, Jung J-S, Park S-H, and Suh H-W (2008) The effect of formalin pretreatment on nicotine-induced antinociceptive effect: the role of mu-opioid receptor in the hippocampus. *Neuroscience* **154**:415–423.
- Leitl MD, Onvani S, Bowers MS, Cheng K, Rice KC, Carlezon WA, Banks ML, and Negus SS (2014) Pain-related depression of the mesolimbic dopamine system in rats: expression, blockade by analgesics, and role of endogenous κ -opioids. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* **39**:614–624.
- Liu X (2013) Positive allosteric modulation of $\alpha 4\beta 2$ nicotinic acetylcholine receptors as a new approach to smoking reduction: evidence from a rat model of nicotine self-administration. *Psychopharmacology (Berl)* **230**:203–213.
- Liu X, Koren AO, Yee SK, Pechnick RN, Poland RE, and London ED (2003) Self-administration of 5-iodo-A-85380, a beta2-selective nicotinic receptor ligand, by operantly trained rats. *Neuroreport* **14**:1503–1505.
- Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Léna C, Le Novère N, de Kerchove d'Exaerde A, Huchet M, Damaj MI, and Changeux JP (1999) Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature* **398**:805–810.
- McLean SL, Grayson B, Idris NF, Lesage AS, Pemberton DJ, Mackie C, and Neill JC (2011) Activation of $\alpha 7$ nicotinic receptors improves phencyclidine-induced deficits in cognitive tasks in rats: implications for therapy of cognitive dysfunction in schizophrenia. *Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol* **21**: 333–343.
- Miller LL, Leitl MD, Banks ML, Blough BE, and Negus SS (2015) Effects of the triple monoamine uptake inhibitor amitriptyline on pain-related depression of behavior and mesolimbic dopamine release in rats. *Pain* **156**:175–184.
- Mishriky BM and Habib AS (2014) Nicotine for postoperative analgesia: a systematic review and meta-analysis. *Anesth Analg* **119**:268–275.
- Mogil JS (2009) Animal models of pain: progress and challenges. *Nat Rev Neurosci* **10**:283–294.
- Mukhin AG, Gündisch D, Horti AG, Koren AO, Tamagnan G, Kimes AS, Chambers J, Vaupel DB, King SL, and Picciotto MR, et al. (2000) 5-Iodo-A-85380, an alpha4-beta2 subtype-selective ligand for nicotinic acetylcholine receptors. *Mol Pharmacol* **57**:642–649.
- Negus SS, Bilskey EJ, Do Carmo GP, and Stevenson GW (2010a) Rationale and methods for assessment of pain-depressed behavior in preclinical assays of pain and analgesia. *Methods Mol Biol* **617**:79–91.
- Negus SS and Miller LL (2014) Intracranial self-stimulation to evaluate abuse potential of drugs. *Pharmacol Rev* **66**:869–917.
- Negus SS, Morrissey EM, Rosenberg M, Cheng K, and Rice KC (2010b) Effects of kappa opioids in an assay of pain-depressed intracranial self-stimulation in rats. *Psychopharmacology (Berl)* **210**:149–159.
- Negus SS, O'Connell R, Morrissey E, Cheng K, and Rice KC (2012) Effects of peripherally restricted κ opioid receptor agonists on pain-related stimulation and depression of behavior in rats. *J Pharmacol Exp Ther* **340**:501–509.
- Negus SS, Vanderah TW, Brandt MR, Bilskey EJ, Becerra L, and Borsook D (2006) Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. *J Pharmacol Exp Ther* **319**:507–514.
- Nirogi R, Goura V, Abraham R, and Jayarajan P (2013) $\alpha 4\beta 2$ neuronal nicotinic receptor ligands (agonist, partial agonist and positive allosteric modulators) as therapeutic prospects for pain. *Eur J Pharmacol* **712**:22–29.
- Nirogi R, Jabaris SL, Jayarajan P, Abraham R, Shanmuganathan D, Rasheed MA, Royapalle PK, and Goura V (2011) Antinociceptive activity of $\alpha 4\beta 2$ neuronal nicotinic receptor agonist A-366833 in experimental models of neuropathic and inflammatory pain. *Eur J Pharmacol* **668**:155–162.
- Panagis G, Kastellakis A, Spyrali C, and Nomikos G (2000) Effects of methyl-lycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology (Berl)* **149**:388–396.
- Pereira Do Carmo G, Stevenson GW, Carlezon WA, and Negus SS (2009) Effects of pain- and analgesia-related manipulations on intracranial self-stimulation in rats: further studies on pain-depressed behavior. *Pain* **144**:170–177.
- Rao TS, Correa LD, Reid RT, and Lloyd GK (1996) Evaluation of anti-nociceptive effects of neuronal nicotinic acetylcholine receptor (NACHR) ligands in the rat tail-flick assay. *Neuropharmacology* **35**:393–405.
- Rode F, Munro G, Holst D, Nielsen EO, Troelsen KB, Timmermann DB, Rønn LCB, and Grunnet M (2012) Positive allosteric modulation of $\alpha 4\beta 2$ nAChR agonist induced behaviour. *Brain Res* **1458**:67–75.
- Rogers DT and Iwamoto ET (1993) Multiple spinal mediators in parenteral nicotine-induced antinociception. *J Pharmacol Exp Ther* **267**:341–349.

- Rosenberg MB, Carroll FI, and Negus SS (2013) Effects of monoamine reuptake inhibitors in assays of acute pain-stimulated and pain-depressed behavior in rats. *J Pain Off J Am Pain Soc* **14**:246–259.
- Rueter LE, Donnelly-Roberts DL, Curzon P, Briggs CA, Anderson DJ, and Bitner RS (2006) A-85380: a pharmacological probe for the preclinical and clinical investigation of the alpha4beta2 neuronal nicotinic acetylcholine receptor. *CNS Drug Rev* **12**:100–112.
- Rueter LE, Kohlhaas KL, Curzon P, Surowy CS, and Meyer MD (2003) Peripheral and central sites of action for A-85380 in the spinal nerve ligation model of neuropathic pain. *Pain* **103**:269–276.
- Sahley TL and Berntson GG (1979) Antinociceptive effects of central and systemic administrations of nicotine in the rat. *Psychopharmacology (Berl)* **65**:279–283.
- Saika F, Kiguchi N, Kobayashi Y, and Kishioka S (2015) Peripheral alpha4beta2 nicotinic acetylcholine receptor signalling attenuates tactile allodynia and thermal hyperalgesia after nerve injury in mice. *Acta Physiol (Oxf)* **213**:462–471.
- Sihver W, Nordberg A, Långström B, Mukhin AG, Koren AO, Kimes AS, and London ED (2000) Development of ligands for in vivo imaging of cerebral nicotinic receptors. *Behav Brain Res* **113**:143–157.
- Spiller K, Xi Z-X, Li X, Ashby CR, Callahan PM, Tehim A, and Gardner EL (2009) Varenicline attenuates nicotine-enhanced brain-stimulation reward by activation of alpha4beta2 nicotinic receptors in rats. *Neuropharmacology* **57**:60–66.
- Tripathi HL, Martin BR, and Aceto MD (1982) Nicotine-induced antinociception in rats and mice: correlation with nicotine brain levels. *J Pharmacol Exp Ther* **221**: 91–96.
- Wang Y, Su D-M, Wang R-H, Liu Y, and Wang H (2005) Antinociceptive effects of choline against acute and inflammatory pain. *Neuroscience* **132**:49–56.
- Weingarten TN, Moeschler SM, Ptaszynski AE, Hooten WM, Beebe TJ, and Warner DO (2008) An assessment of the association between smoking status, pain intensity, and functional interference in patients with chronic pain. *Pain Physician* **11**:643–653.
- Whiteaker P, Garcha HS, Wonnacott S, and Stolerman IP (1995) Locomotor activation and dopamine release produced by nicotine and isoarecolone in rats. *Br J Pharmacol* **116**:2097–2105.
- Whiteside GT, Adedoyin A, and Leventhal L (2008) Predictive validity of animal pain models? A comparison of the pharmacokinetic-pharmacodynamic relationship for pain drugs in rats and humans. *Neuropharmacology* **54**:767–775.
- Wieskopf JS, Mathur J, Limapichat W, Post MR, Al-Qazzaz M, Sorge RE, Martin LJ, Zaykin DV, Smith SB, and Freitas K, et al. (2015) The nicotinic $\alpha 6$ subunit gene determines variability in chronic pain sensitivity via cross-inhibition of P2X2/3 receptors. *Sci Transl Med* **7**:287ra72.
- Young T, Wittenauer S, Parker R, and Vincler M (2008) Peripheral nerve injury alters spinal nicotinic acetylcholine receptor pharmacology. *Eur J Pharmacol* **590**: 163–169.

Address correspondence to: S. Stevens Negus, Department of Pharmacology and Toxicology, Virginia Commonwealth University, 410 North 12th Street, PO Box 980613, Richmond, VA 23298. E-mail: ssnegus@vcu.edu