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$\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonists with low intrinsic efficacy have antidepressant-like properties

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Abstract

Previous studies have suggested that treatment with antagonists or partial agonists of nicotinic acetylcholine receptors containing the $\beta 2$ subunit ($\beta 2^*$ nAChRs) results in antidepressant-like effects. In the current study we tested 3 novel compounds with different affinity and functional efficacy at $\alpha 4\beta 2^*$ nAChRs, which were synthesized as part of nAChR discovery projects at Pfizer in the tail suspension, forced swim and novelty-suppressed feeding tests of antidepressant efficacy. All compounds tested reduced immobility in the forced swim test and one of the compounds also reduced immobility in the tail suspension test. All the compounds appeared to affect food intake on their own, with 2 compounds reducing feeding significantly in the home cage, precluding a clear interpretation of the results in the novelty-suppressed feeding test. None of the compounds altered locomotor activity at the doses and time points used here. Therefore, a subset of these compounds has pharmacological and behavioral properties that demonstrate the potential of nicotinic compounds as a treatment of mood disorders. Further development of nicotinic-based antidepressants should focus on increasing nAChR subtype selectivity to obtain consistent antidepressant properties with an acceptable side effect profile.

Keywords

Nicotinic acetylcholine receptors; partial agonists; depression; forced swim test; tail suspension test; novelty-suppressed feeding; mice

INTRODUCTION

Individuals with major depressive disorder are twice as likely to smoke as the general population, demonstrating that there is significant co-morbidity between smoking and depression-related disorders and smoking cessation can exacerbate symptoms of depression (Glassman et al., 1990). Conversely, use of the nicotine patch can reduce symptoms of depression in people who do not smoke (Salin-Pascual et al., 1995) and animal studies suggest that chronic nicotine administration can result in antidepressant-like phenotypes in various models of antidepressant efficacy (Semba et al., 1998; Djuric et al., 1999; Tizabi et al., 1999). This has led to the idea that smokers may use the nicotine in tobacco to self-medicate depressive symptoms.

Heightened cholinergic tone following administration of an acetylcholinesterase inhibitor to human subjects can result in increased symptoms of depression (Janowsky et al., 1972; Janowsky et al., 1974) and increased sensitivity to cholinergic agents has been reported in depressed patients (reviewed in Janowsky et al., 1994). It was initially believed that heightened cholinergic tone was acting mainly through muscarinic acetylcholine receptors, and muscarinic blockers can show antidepressant-like effects (Furey and Drevets, 2006); however, increased cholinergic tone will also affect the function of nicotinic acetylcholine receptors (nAChRs) and a growing body of studies suggests that modulation of nAChR function is associated with antidepressant-like effects (for reviews, see Picciotto et al., 2008; Mineur and Picciotto, 2009, 2010). The idea that increased ACh signaling in the brain may contribute to depressive symptoms seemed at odds with the finding that chronic nicotine administration can also be antidepressant. These observations can be reconciled when taking into account that nicotine initially activates, but subsequently desensitizes nAChRs, which leads to a persistent decrease in endogenous cholinergic signaling following chronic nicotine exposure (Quick and Lester, 2002; Picciotto et al., 2008; Mineur and Picciotto, 2010). Thus, limiting ACh-induced nAChR activation may be expected to have antidepressant-like properties. We previously showed that the antagonist mecamylamine, a non-competitive, non-selective nAChR channel blocker, has antidepressant-like effects in several animal models of antidepressant efficacy (Caldarone et al., 2004; Rabenstein et al., 2006). Recent clinical studies have confirmed the antidepressant potential of mecamylamine (George et al., 2008; Dunbar et al., 2007) and of an enantiomer, (+)-mecamylamine (Dunbar, 2009), in patients with major depression previously unresponsive to a selective serotonin reuptake inhibitor (SSRI).

Experiments using knockout mice (Rabenstein et al., 2006) and pharmacological studies with selective nicotinic compounds (Mineur et al., 2007c; Mineur et al., 2009a) suggest that reduced activity at high affinity $\alpha 4 \beta 2$ * nAChRs mediates the antidepressant-like properties of nicotinic antagonists and partial agonists (* denotes potential additional subunits *in vivo* and when subtypes are tested *in vitro* with defined subunits, no * is used). Varenicline is an $\alpha 4 \beta 2$ nAChR partial agonist (Coe et al., 2005a; Rollema et al., 2007a) that also induces antidepressant-like behavior in the forced swim test in two different mouse strains and augments the effect of the SSRI sertraline in the forced swim test in CD-1 mice (Rollema et al., 2009). Derivatives of another nAChR partial agonist, cytisine, have also been shown to exhibit antidepressant-like properties (Mineur et al., 2009a). Here we examined the effects of three compounds that were identified as high affinity/low efficacy $\alpha 4 \beta 2$ nAChR partial agonists, in mouse models of antidepressant efficacy to evaluate their potential for clinical development.

MATERIALS AND METHODS

Drugs

CP-360288 (3-bromocytisine), CP-601927 and CP-601932 are $\alpha 4 \beta 2$ nAChR partial agonists identified at Pfizer as part of drug discovery programs targeting nAChR partial agonists for smoking cessation (Coe et al., 2005a; Coe et al., 2005b; Rollema et al., 2007b; Coe et al., 2009). Details of the synthesis, preclinical pharmacology and pharmacokinetics of these compounds are reported elsewhere (Coe et al., 2005b; Shaffer et al., 2009; Coe et al., 2009; Shaffer et al., 2010; Chatterjee et al., 2010). *In vitro* binding affinities and relative agonist efficacies at $\alpha 4 \beta 2$ and $\alpha 3 \beta 4$ nAChR subtypes are described in Table 1. We focused on these two receptor subtypes because $\alpha 4 \beta 2$ * nAChRs are critical for the antidepressant-like properties of nicotinic antagonists (Caldarone et al., 2004; Rabenstein et al., 2006; Mineur et al., 2007c) and because $\alpha 3 \beta 4$ nAChRs can contribute to increased anxiety-like behavior and decrease feeding (Salas et al., 2004; Mineur et al., 2007a), which are potential confounding factors for the paradigms used in this study.

Doses were selected based both on drug affinities for nAChR subtypes and on previous results obtained with varenicline and cytosine (Mineur et al., 2007c; Mineur et al., 2009b), while upper dose limits were determined by observed adverse effects, such as arched back or decreased activity in the home cage. All compounds were dosed intraperitoneally (i.p.) in a volume of 10 ml/kg in phosphate buffered saline (Saline) and doses are expressed as the active base.

CP-601927 and CP-601932 were dosed at 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1.0 mg/kg and 1.5 mg/kg. Since initial high doses of 3-bromocytisine significantly altered motility of mice, this compound was administered at 0.075 mg/kg, 0.125 mg/kg, 0.25 mg/kg, 0.75 mg/kg, and 1.0 mg/kg. Fluoxetine was purchased from Spectrum Chemicals and used at 10 mg/kg as a positive control following the same regimen of testing as the nicotinic compounds. Saline or drug was injected i.p. 30 min prior to the tail suspension test or the forced swim test, and the two tests were separated by 1 week. After the forced swim test, Saline or drug was injected once daily (between 9 and 11 AM) for 15 days, after which mice were tested in the novelty-suppressed feeding paradigm. Novelty-suppressed feeding was performed in the morning before the usual daily injection to avoid any acute effects of the drugs. For each test, experiments were performed under similar conditions and in parallel with fluoxetine (10 mg/kg, ip) and varenicline (1.5 mg/kg) as positive controls (Rollema et al., 2009).

Animals

C57BL/6J male mice (240 mice, 10 to 12 weeks of age) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Upon arrival, animals were split into groups of 5 mice, and were randomly assigned to a specific treatment group ($n = 10$ for all groups). Mice were allowed to acclimate for at least one week before beginning experiments. Three days before the experiments, mice were marked with a permanent marker at the base of their tail and subsequently weighed. This was repeated each week. All *in vivo* procedures were conducted in compliance with a protocol approved by the Yale Animal Care and Use Committee.

Behavioral assays

Since previous studies have demonstrated that subjecting a single cohort of C57BL/6J mice to a battery of tests of antidepressant efficacy yields similar results to performing each test in a separate cohort of mice (Rabenstein et al., 2006; Mineur et al., 2007b; Mineur et al., 2007c) and reduces the number of animals necessary for behavioral testing, the same mice were used in a battery of behavioral tests. Each individual mouse received the same compound at a single concentration in each test (i.e., if a mouse received compound A at 1 mg/kg, that animal received one injection before tail suspension testing, one injection before forced swim testing and then 1 daily injection for 15 days followed by novelty-suppressed feeding testing). The least stressful test was performed first, as has been suggested for behavioral phenotyping (Crawley et al., 1997; Crawley, 2008). The schedule of behavioral testing was as follows: Day 1: tail suspension test; Day 8: forced-swim test 1; Day 23: novelty-suppressed feeding test; Day 26: locomotor activity (20 min). Both the tail suspension and the forced swim tests are sensitive to acute antidepressant administration (Cryan and Holmes, 2005), whereas the novelty-suppressed feeding test is only sensitive to chronic treatment with antidepressants (Dulawa and Hen, 2005).

For all procedures mice were transferred to the testing room at least 30 min before the beginning of testing. Experimenters were blind to treatment. Mice were only returned to the colony room once all animals had completed the day's testing. Four cohorts of 60 mice were tested in these studies.

Tail suspension test—For tail suspension testing mice were gently suspended by the tail and scored for the time spent immobile over the 6 min test as described previously (Mineur et al., 2007b). Immobility was defined as no movement except for respiration. After completion of the test, mice were returned to a holding cage until all cage-mates were tested.

Forced swim test—Mice were placed in clear glass beakers filled with 15 cm water (~25°C) for 15 min with care taken not to put the nose of the mouse below water level when initially placed in the water (Mineur et al., 2007c; Mineur et al., 2009b). Mice were scored for time spent immobile (immobility was defined as a minimal amount of movement made by the mouse to stay afloat). After testing, each mouse was placed in a heated holding cage (30–35°C) with bedding covered by a paper towel and then returned to the holding room.

Novelty-suppressed feeding test—Each compound was injected once daily for 15 days (each mouse received only one dose of a compound). After treatment, mice were weighed and food was removed from the cage. Twenty four hours later, mice were transferred to the testing room, weighed again, placed in a clean holding cage and allowed to habituate for at least 30 min. Mice were then placed in a corner of the testing apparatus, consisting of a clear Plexiglas enclosure (40 × 40 × 17 cm), with a lid and 2 cm of corn cob bedding. A small piece of chow (about 0.5 cm in diameter) was placed in the center of the arena on a piece of white circular filter paper (9.5 cm in diameter). After initiation of testing, the time to the first feeding event was measured. Upon initiation of feeding behavior, the mouse was removed from the testing area and placed alone for 5 min in its original home cage with a weighed piece of lab chow. After 5 min, the lab chow pellet was weighed again to measure home cage food consumption. Once all cage-mates were tested, mice were returned to their home cage.

Locomotor activity—Three days after the final test of antidepressant-efficacy, mice were tested for locomotor activity 30 min after drug treatment, for 20 min. Each animal was placed in a clean transparent rat cage (48 × 22 × 18 cm) and locomotor activity was recorded as beam breaks using the Optomax system (Columbus instruments, Columbus, Ohio, USA). Subjects were returned to their home cage at the end of the session.

Statistical analyses

Data were analyzed using one-way analyses of variance (ANOVA) with “dose” as a between-subject factor. When relevant, posthoc analyses were performed by t-tests with Tukey-Kramer corrections for multiple comparisons. For analysis of locomotor activity data, we performed a repeated-measure ANOVA with “2-min time bin” as a within-subject factor, and “treatment” as a between-subject factor. A *p* value < 0.05 was considered significant.

RESULTS

Properties of test compounds (Table 1)

Table 1 summarizes binding affinities and intrinsic activities of 3-bromocytisine, CP-601927 and CP-601932 at $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs as compared to nicotine, cytosine and varenicline. The test compounds have high affinity and relatively low efficacy for $\alpha 4\beta 2$ nAChRs, while potencies and efficacies at $\alpha 3\beta 4$ nAChRs vary. All compounds used here had low binding affinity for $\alpha 1$ containing ($K_i \geq 0.5 \mu\text{M}$) and $\alpha 7$ nAChRs ($K_i > 1 \mu\text{M}$), except for 3-bromocytisine, which is a full agonist at $\alpha 7$ nAChRs with a K_i value of 29 nM. Finally, all compounds had good brain penetration after systemic administration (Coe et al. 2009; Rollema et al. 2010). At the highest doses used for testing of antidepressant-like efficacy, none of the test compounds altered locomotor activity, indicating that results were not confounded by stimulant effects of these compounds ($F(2, 18) = 0.32$, $p = 0.99$; Fig. 1).

Tail suspension (Fig. 2)

3-bromocytisine or CP-601927 had no overall effect in the tail suspension test ($F(4, 44) = 0.403$, $p=0.8$ and $F(4, 40)=1.96$, $p=0.12$, respectively, Fig. 2A and B). However, CP-601932 induced an overall effect on the reduction of time spent immobile ($F(4, 45) = 5.38$, $p = 0.012$; Fig. 1C). Posthoc analyses indicated that compared to the control group, only the 1.5 mg/kg dose of CP-601932 was significantly different from saline in reducing time spent immobile ($p<0.001$) while other doses resulted only in non-significant effects on immobility. As positive controls, both varenicline (1.5 mg/kg) and fluoxetine (10 mg/kg) induced a significant decrease in immobility ($F(1, 18) = 4.72$, $p = 0.045$ and $F(1, 18) = 0.89$, $p = 0.0007$, respectively).

Forced swim test (Fig. 3)

3-Bromocytisine showed an overall effect across doses in the forced swim test ($F(4, 34)=7.44$, $p=0.0002$, Fig. 3A). In comparison to the control group, posthoc analyses revealed a significant reduction in the time spent immobile at 0.075 mg/kg ($p<0.001$), 0.125 mg/kg ($p=0.0005$) and 1 mg/kg ($p=0.0001$), while the decrease in immobility time at 0.25 mg/kg did not reach statistical significance ($p=0.093$).

CP-601927 had an overall treatment effect on the reduction of time spent immobile ($F(4, 44) = 12.6$, $p<0.0001$; Fig. 3B) in the forced swim test. Posthoc analyses indicated that compared to the control group, CP-601927 significantly reduced the time spent immobile for all doses tested compared to saline-treated animals: 0.25 mg/kg ($p=0.0003$), 0.75 mg/kg ($p<0.0001$), 1 mg/kg ($p<0.0001$) and 1.5 mg/kg ($p<0.0001$).

CP-601932 caused an overall reduction in the time spent immobile in the forced swim test ($F(4, 44) = 25.63$, $p<0.0001$; Fig. 3B). Posthoc analyses indicated that all doses tested significantly reduced the time spent immobile ($p's<0.0001$) compared to the control group; however, the 1 mg/kg dose showed a less efficacious response than the other doses (0.75 mg/kg vs. 1 mg/kg: $p=0.0003$; 1 mg/kg vs. 1.5 mg/kg: $p<0.0001$).

As positive controls, both varenicline (1.5 mg/kg) and fluoxetine (10 mg/kg) induced a significant decrease in immobility ($F(1, 17) = 141.7$, $p < 0.0001$ and $F(1, 17) = 6.7$, $p = 0.01$, respectively).

Novelty-suppressed feeding test (Fig. 4)

3-Bromocytisine caused no overall reduction in the time to first feeding episode in the open field ($F(5, 31) = 1.1$, $p = 0.38$; Fig. 4A). This compound was also found to reduce food intake in the home cage ($F(5, 31) = 10.23$, $p<0.001$; Fig. 4B).

CP-601927 treatment led to an overall increase in the time to first feeding episode in the open field ($F(4, 39) = 4.86$, $p = 0.0028$; Fig. 4C) in the novelty-suppressed feeding test, although it did not reduce food intake overall in the home cage ($F(4, 39) = 1.05$, $p = 0.39$; Fig. 4D). Posthoc analyses showed that 1.0 mg/kg and 1.5 mg/kg significantly increased the time to first feed ($p's < 0.01$) compared to the control group, mainly because most animals did not eat during the test.

CP-601932 produced an overall increase in the time to first feeding episode in the open field ($F(4, 44) = 8.46$, $p < 0.001$; Fig. 4E), while reducing food intake in the home cage ($F(4, 44) = 5.71$, $p = 0.0009$; Fig. 4F). Posthoc analyses showed that every dose significantly increased the time to the first feeding episode ($p's < 0.001$) compared to the control group, but animals did not eat at all after receiving most doses.

While fluoxetine significantly decreased the time to first feeding episode ($F(1, 19) = 7.79$, $p = 0.007$), varenicline was unable to induce the same effects ($F < 1$), most likely due to a significant decrease in food intake in home cage ($F(1, 17) = 4.78$, $p = 0.04$).

DISCUSSION

The current set of studies investigated the antidepressant-like effects of three novel $\alpha 4\beta 2$ nAChR partial agonists: a bromo-derivative of cytosine, 3-bromocytisine, and the enantiomeric pair, CP-601927 and CP-601932, which have been in development as smoking cessation aids.

3-Bromocytisine demonstrates extremely high binding affinity for $\alpha 4\beta 2$ nAChRs ($K_i = 0.2$ nM), as well as for $\alpha 3\beta 4$ nAChRs ($K_i = 3.6$ nM), which are highly expressed in the autonomic ganglia. Potentially due to high *in vivo* potency, this compound was not well tolerated at doses of 1 mg/kg and above. These high 3-bromocytisine doses caused adverse effects in mice, including abnormal movements. None of the test doses of 3-bromocytisine induced an antidepressant-like effect in the tail suspension test, but, in agreement with its high potency, very low doses of 3-bromocytisine decreased immobility in the forced swim test. Finally, although chronic treatment with classical antidepressants generally reduces the time to first feed in an open field (Dulawa and Hen, 2005), 3-bromocytisine was ineffective in decreasing latency to feed in the novelty-suppressed feeding test. 3-bromocytisine did not induce any obvious signs of discomfort in mice at any of the doses tested, however. Consistent with an anorexogenic effect of 3-bromocytisine, mice treated with this compound showed reduced home-cage food intake. This is consistent with previous studies of nicotinic drugs, since nicotinic agents, including cytosine, decrease appetite (Hughes and Hatsukami, 1997; Filozof et al., 2004). A decrease in appetite would confound any interpretation of changes seen in the novelty-suppressed feeding test. In addition, the high affinity of the compound for $\alpha 3\beta 4$ nAChRs could contribute to an anxiogenic effect of this compound (Salas et al., 2003).

The discrepancy between the results of the tail suspension and the forced swim tests was not anticipated, since performance in both tests is sensitive to treatment with currently used antidepressants including fluoxetine. The origin of these discrepancies is unknown and is also not consistent with PK related exposure differences between the models, as time points are virtually identical. It is conceivable that each model is responsive to distinct pharmacological properties of antidepressant drugs that are not well understood. Also, other peripheral effects (including vasoconstriction and hypothermia) may be confounds in the different outcomes measured here. Overall, the mixed effects observed across the different behavioral tests performed here, along with the poorly tolerated effects at higher doses, makes 3-bromocytisine unsuitable as a lead compound for developing a treatment of mood disorders.

While 3-bromocytisine showed some antidepressant-like properties in this study, peripheral administration of the 5-bromo analog, 5-bromocytisine, that has comparable binding affinity for the $\alpha 4\beta 2$ nAChR ($K_i = 0.3$ nM), but lower agonist efficacy (17%) compared to 3-bromocytisine, did not induce significant effects in tests of antidepressant efficacy when administered peripherally. This was most likely due to poor brain penetration, since central administration resulted in decreased immobility in the forced swim test (Mineur et al., 2009a). It is also possible that lower efficacy at $\alpha 4\beta 2$ nAChRs contributed to the difference in response to the 5-bromo- compared to the 3-bromo analog (Coe et al, 2009).

Compared to cytosine, another nAChR partial agonists previously shown to induce significant antidepressant-like effects in mice (Mineur et al., 2007b; Rollema et al., 2009),

CP-601927 binds with lower affinity to $\alpha 4\beta 2$ nAChRs and also has lower agonist efficacy (Table 1). Thus, CP-601927 could decrease cholinergic signaling as a result of its reduced efficacy or via desensitization of $\alpha 4\beta 2^*$ nAChRs. In the tail suspension test, an observable decrease in immobility was observed, with a peak at 0.75 mg/kg, although none of the doses tested induced a significant difference. In contrast, treatment with CP-601927 strongly decreased time spent immobile in the forced swim test at all doses tested. The discrepancy in effects of CP-601927 in the two tests may be related to underlying differences in the two paradigms described for 3-bromocytisine above. Also, while the compounds used here have varying affinity for nAChRs, it is difficult to relate these affinities to their antidepressant-like effects without more precise data on whether the compounds have differential penetration into the brain. However, it is also worth noting that the experimental design involved 4 different concentrations for each compound, along with saline and fluoxetine, that resulted in stringent statistical corrections for multiple group comparisons. Thus, while the effects of CP-601927 were smaller in the tail suspension test as compared to those observed in the forced swim test, a non-significant observable effect on immobility was observed. As with 3-bromocytisine, the effect of CP-601927 on novelty-suppressed feeding appears to be confounded by anorexigenic effects of the compound. The ability of nicotinic agents to decrease food intake may have some value in drug development, since most classical antidepressants used currently induce weight gain (Ginsberg, 2009). It is worth noting, however, that the decrease in home cage feeding observed with CP-601927 was not closely correlated with the decreased time to the first feeding episode in the open field, indicating that other factors may also be involved in the novelty-suppressed feeding effects. For instance, acute nicotine treatment can be anxiogenic (File et al., 2002; Tucci et al., 2002) and previous studies indicate that acute injection of a nicotinic partial agonist can also be anxiogenic in the light/dark test in mice (Mineur et al., 2007c). The high efficacy of this compound for $\alpha 3\beta 4$ nAChRs (80%) may also contribute to an anxiogenic effect, which could increase the time for the mice to reach the center of the open field and eat.

CP-601932 has only 2% agonist efficacy at $\alpha 4\beta 2$ nAChRs as measured by *in vitro* physiological assays and is therefore a functional antagonist with lower binding affinity for $\alpha 4\beta 2$ nAChRs compared to the other compounds tested in this study. This compound also has equivalent affinity for $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs (21 nM), with significant efficacy (30%) at the $\alpha 3\beta 4$ subtype; thus, this compound may affect both nAChR subtypes (Chatterjee et al, 2010). In the tail suspension test, CP-601932 dose-dependently decreased immobility and this reached significance at the highest dose tested. Similar effects of CP-601932 were observed in the forced swim test, but significance was reached at the lowest concentration tested. Finally, the results in the novelty-suppressed feeding test were inconclusive, as has been discussed for the two other compounds tested in this study, because CP-601932 dose-dependently decreased feeding in the home cage and this was negatively correlated with the time to first feed in the open field. This strongly suggests that decreased appetite was a primary factor in the effects of CP-601932 on behavior in this paradigm.

In conclusion, the current results provide further support for previously published studies indicating that decreasing signaling at nAChRs can induce antidepressant-like responses in mouse models of antidepressant efficacy. Blockade of $\alpha 4\beta 2^*$ nAChRs can result in antidepressant-like effects; but, reducing cholinergic signaling via $\alpha 4\beta 2^*$ nAChRs using partial agonists that compete with endogenous ACh signaling may be more desirable than complete blockade of these receptors. It is not yet possible to determine how much of a decrease in cholinergic tone is necessary to achieve an antidepressant-like response, but it is very likely that this depends on the degree of cholinergic activity present during the behavioral evaluation and the complement of cholinergic receptors available for signaling in the particular individual tested. Although it is difficult to quantify the effects of nicotinic compounds across animal models that may have differential activation of the cholinergic

system, nicotinic agonists appear to be less effective than antagonists of high affinity nAChR receptors in mouse models of antidepressant efficacy (Andreasen et al., 2008; Andreasen and Redrobe, 2009). In addition, complete blockade of nAChRs may lead to more, or different, side effects than partial agonism. All test compounds display high affinity for $\alpha 4\beta 2$ nAChRs and consistency in performance in the forced swim test but varying effects at $\alpha 3\beta 4$ nAChRs and inconsistency in tolerability at higher doses. Thus, subtype selectivity for the $\alpha 4\beta 2$ class of nAChRs should also be a key element in the development of nicotinic compounds to be used for treatment of depression, since modulation of other nAChR subtypes may result in increased anxiety and perturbation of feeding (Salas et al., 2003; Mineur et al., 2007a). Thus, the challenge for further development of nicotinic-based antidepressants will be to increase nicotinic receptor specificity along with a moderate decrease in activity of endogenous cholinergic activation of $\alpha 4\beta 2^*$ nAChRs in order to obtain consistent antidepressant properties with limited side effects.

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Figure 1. Locomotor activity

Locomotor activity was measured as the number of beam breaks for mice treated with 3-bromocytisine, CP-601927, and CP-601932. The X-axis represents 2-min time bins. The Y-axis represents number of beam breaks. Error bars represent SEM. N=10 for each treatment group.

Figure 2. Tail suspension test

Total time spent immobile in the tail suspension by C57BL/6J male mice treated with (A) 3-bromocytisine, (B) CP-601927 and (C) CP-601932 at several concentrations. Saline is used as the negative control group. (D) Treatment with fluoxetine at 10 mg/kg (Flx 10) and varenicline at 1.5 mg/kg (Varen 1.5). Error bars represent SEM. * $P < 0.05$, *** $P < 0.001$. $N=10$ for each treatment group.

Figure 3. Forced swim test

Total time spent immobile in the forced swim tests by C57BL/6J male mice treated with (A) 3-bromocytisine, (B) CP-601927 and (C) CP-601932 at several concentrations. Saline is used as the negative control group. (D) Treatment with fluoxetine at 10 mg/kg (Flx 10) and varenicline at 1.5 mg/kg (Varen 1.5; NOTE: these data were previously published in (Rollema et al., 2009)). Error bars represent SEM. ** $P < 0.01$, *** $P < 0.001$. $N=10$ for each treatment group.

Figure 4. Novelty-suppressed feeding

Time to first feeding episode in the open field and home cage food intake by C57BL/6J male mice treated with (A and B) 3-bromocytisine, (C and D) CP-601927 and (E and F) CP-601932 at several concentrations. Saline is used as the negative control group and (G and H) treatment with fluoxetine at 10 mg/kg (Flx 10) and varenicline at 1.5 mg/kg (Varen 1.5) are used as comparison controls. Error bars represent SEM. * P < 0.05, ** P < 0.01, *** P < 0.001. N=10 for each treatment group.

TABLE 1
(%) and structures of nicotine and $\alpha 4 \beta 2$ nAChR partial agonists

Varenicline ¹	Cytisine ¹	3-Bromocytisine ²	CP-601927 ²	CP-601932 ⁴
0.4 ± 0.1 22 $\pm$ 2.5	2.0 ± 0.2 6.5 $\pm$ 0.2	0.2 ± 0.2 50 $\pm$ 12 ^a	1.2 ± 0.2 15.6 $\pm$ 1.6	21 ± 19 ND ⁵
86 ± 16 93.5 $\pm$ 1.4	480 ± 63 100 $\pm$ 3.8 ^{3,a}	3.6 ± 1.2 ND	102 ± 22 79.9 $\pm$ 12.5	21 ± 28 30.2 $\pm$ 3.4

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cells.

highest test concentration