Pharmacology, Biochemistry and Behavior 179 (2019) 109–112

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Oral sazetidine-A, a selective α4β2* nicotinic receptor desensitizing agent, reduces nicotine self-administration in rats

Amir H. Rezvani[a], Corinne Wells[a], Susan Slade[a], Yingxian Xiao[b], Kenneth J. Kellar[b], Edward D. Levin[a,*]

[a] Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, USA
[b] Department of Pharmacology & Physiology, Georgetown University School of Medicine, USA

ABSTRACT

Sazetidine-A selectively desensitizes α4β2 nicotinic receptors and also has partial agonist effects. We have shown that subcutaneous acute and repeated injections as well as chronic infusions of sazetidine-A significantly reduce intravenous (IV) nicotine self-administration in rats. To further investigate the promise of sazetidine-A as a smoking cessation aid, it is important to determine sazetidine-A effects with oral administration and the time-effect function for its action on nicotine self-administration. Young adult female Sprague-Dawley rats were trained to self-administer IV nicotine at the benchmark dose of 0.03 mg/kg/infusion dose in an operant FR1 schedule in 45-min sessions. After five sessions of training, they were tested for the effects of acute oral doses of sazetidine-A (0, 0.3, 1 and 3 mg/kg) given 30 min before testing. To determine the time-effect function, these rats were administered 0 or 3 mg/kg of sazetidine-A 1, 2, 4 or 23 h before the onset of testing. Our previous study showed that with subcutaneous injections, only 3 mg/kg of sazetidine-A significantly reduced nicotine self-administration, however, with oral administration of sazetidine-A lower dose of 1 mg/kg was also effective in reducing nicotine intake. A similar effect was seen in the time-effect study with 3 mg/kg of oral sazetidine-A causing a significant reduction in nicotine self-administration across all the time points of 1, 2, 4 or 23 h after oral administration. These results advance the development of sazetidine-A as a possible aid for smoking cessation by showing effectiveness with oral administration and persistence of the effect over the course of a day.

1. Introduction

Tobacco addiction continues to be a major public health problem worldwide. The seemingly simple act of smoking cessation can have substantial health benefits. However, successful long-term smoking cessation is surprisingly difficult to achieve even with pharmaceutical assistance. The approved tobacco/smoking cessation treatments including various types of nicotine replacement, bupropion and varenicline provide some improvement in cessation rates (Fiore et al., 1994; Jorenby et al., 2006; Levin et al., 1994; Rollema et al., 2007), but there is still considerable room for improvement. New, more effective treatments are needed. It has become apparent that no single smoking cessation aid can help all smokers to quit. A greater diversity of effective treatments will help with the tailoring of the most effective therapy for the needs of the diverse array of smokers. Many current treatments act on neuronal nicotinic acetylcholine receptors. Nicotine has actions to both activate and desensitize nicotinic receptors (Katz and Thesleff, 1957; Quick and Lester, 2002). The relative roles of nicotinic receptor activation and desensitization in reinforcement and self-administration of nicotine are not fully understood.

Current nicotinic-based treatments do successfully reduce nicotine self-administration over the short-term, but fail to maintain long-term success in the majority of smokers leading to a high rate of relapse. Varenicline, a partial α4β2 agonist, has been shown to reduce smoking relative to placebo and bupropion (Jorenby et al., 2006; Rollema et al., 2007), but it has some adverse side effects, including nausea, abnormal dreams, and much more rarely possible exacerbation of psychiatric symptoms (Freedman, 2007; Morstad et al., 2008). These side effects may be due to effects at non-α4β2 nicotinic receptors, such as α3β4* and/or α7 subtypes. A drug that more selectively targets the α4β2 receptor may be less likely to produce the adverse side effects and consequently be more effective. One possibility is α4β2-selective drugs such as sazetidine-A.

Sazetidine-A is a nicotinic partial agonist with a high affinity and great selectivity for α4β2 nicotinic receptors. It desensitizes α4β2 receptors with long lasting effects (Xiao et al., 2006). Systemic administration of sazetidine-A has been found in several studies to be effective in reducing nicotine self-administration with both acute and repeated administration at 3 mg/kg, with only modest effects on locomotion and food-motivated responding (Johnson et al., 2012; Levin et al., 2010; Rezvani et al., 2010). Interestingly, it has also been shown to significantly reduce alcohol intake in alcohol preferring rats (Rezvani

* Corresponding author at: Department of Psychiatry and Behavioral Sciences, PO Box 104790, Duke University Medical Center, Durham, NC 27710, USA.
E-mail address: edlevin@duke.edu (E.D. Levin).

https://doi.org/10.1016/j.pbb.2019.02.007
Received 31 October 2018; Received in revised form 26 December 2018; Accepted 18 February 2019
Available online 19 February 2019
0091-3057/

et al., 2010). Moreover, while chronic administration of nicotine and varenicline up-regulate α4β2 nicotinic receptors in rodent brain, an effect that may be important to underlying addiction mechanisms at the cellular level, sazetidine-A does not up-regulate these receptors (Hussmann et al., 2012).

The goal of the present study was to evaluate the efficacy of oral administration of sazetidine-A in reducing nicotine self-administration. In addition, the time-effect function was assessed to determine the persistence of effectiveness of oral sazetidine-A in reducing nicotine self-administration throughout the day after dosing.

2. Methods

2.1. Subjects

Young adult female ($N = 20$) Sprague-Dawley rats (Taconic Lab, Germantown, NY, USA) were used in the present study. Females were used to facilitate comparison with our previous studies of the acute effects of sazetidine-A given by the sc route and effects on nicotine self-administration. Animals were individually housed in a temperature-controlled vivarium room located adjacent to the nicotine self-administration testing room. Animals were maintained on a 12:12 reverse light-dark cycle (lights off at 7.00 a.m.) so that experimental sessions occurred during the active phase of the rats' diurnal cycle. Animals were given ad libitum access to water at all times excluding experimental sessions, and were fed daily 20–30 min after the completion of their experimental session. The rats were maintained at roughly 85% of their free-feeding weight, and healthily gained weight throughout the study. At the end of each study catheter patency tests were conducted and animals with obstructed catheters were dropped from analysis. All procedures were approved by the IACUC at Duke University.

2.2. Drug treatment

Sazetidine-A [6-(5((*S*)-azetidin-2-yl)methoxy)pyridine-3-yl-1-ol] was synthesized as described previously (Xiao et al., 2006) and supplied by RTI International (Durham, NC, USA) via National Institute on Drug Abuse (NIDA). The drug was dissolved in water and delivered by oral gavage in doses of 0, 0.3, 1 and 3 mg/kg in a volume of 4 ml/kg twice in the dose-effect study. Water was the vehicle control. Testing the effects of different doses of sazetidine-A in the 45-min session began 30-min after the oral administration of the drug. To evaluate the persistence effects of sazetidine, in a seperate time-effect experiment, the effects of sazetidine-A were tested at 1-h, 2-h, 4-h or 23-h after oral administration twice.

Nicotine bitartrate solutions were prepared weekly in sterilized isotonic saline. The dose of nicotine used for self-administration (0.03 mg/kg/infusion) was calculated as a function of the nicotine free base molecular weight. The pH of the nicotine solution was adjusted to 7.0 using NaOH and the solution was filtered in a Nalgene filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. Between sessions all nicotine was kept in a dark refrigerator.

2.3. Behavioral procedures

Before the start of nicotine self-administration sessions, all animals were trained to lever press in a standard dual-lever experimental chamber (Med Associates, St. Albans, VT, USA) for food reinforcement. Each chamber was equipped with two levers (one active, one inactive), two cue lights located directly above each lever, a house light, and a tone generator. After lever pressing was established, animals experienced three sessions of lever pressing for food under a fixed ratio (FR) 1 schedule of reinforcement. Following the completion of their final training session with food reinforcement, animals were anesthetized with ketamine (60 mg/kg) and domitor (15 mg/kg) and a catheter (Strategic Application Inc., Libertyville, IL, USA) was implanted into their jugular vein. The jugular catheter was attached to a harness that could be tethered to the infusion pump during experimental sessions. Animals were given a minimum of 24 h to recover from the surgery before experiencing nicotine self-administration sessions.

Following recovery from the surgery, animals were given 5 sessions in the operant chambers where a correct lever press resulted in the delivery of a nicotine infusion on a fixed ratio (FR) 1 schedule of reinforcement, and the activation of a feedback tone for 0.05 s. Each infusion was followed by a one-min period time out where the cue lights went out, the house light came on and correct responses were recorded but not reinforced. Each nicotine self-administration session lasted for 45-min. After the initial 5 sessions of nicotine self-administration training the rats were tested for the effects of oral sazetidine-A administration.

To keep the catheters patent, they were flushed daily before the experimental sessions with a 100 U/ml heparinized saline solution. After the completion of a test session, nicotine remaining in the port was removed and a 0.3-ml sterile lock solution containing 500 U/ml of heparinized saline and 8 mg/ml of gentamicin (American Pharmaceutical Partners, Schaumberg, IL, USA) was infused.

2.4. Data analysis

The data were evaluated with a within subject design analysis of variance. Drug-dose, repeated test phase and timing were within subjects factors. An alpha level of $p < 0.05$ was used to determine statistical significance. Significant interactions were followed by tests of the simple main effects.

3. Results

3.1. Pretraining

Over the course of the five sessions of training, the rats increased nicotine self-administration from 3.15 ± 0.89 infusions during the first session to 6.05 ± 1.50 infusions per session during the fifth session.

3.2. Sazetidine-A oral dose-effect function

Oral administration of sazetidine-A significantly reduced nicotine self-administration. Planned comparisons of control levels vs. each of the dose levels showed that the doses of 1 mg/kg ($F(1,57) = 6.28$, $p < 0.025$) and 3 mg/kg ($F(1,57) = 4.78$, $p < 0.05$) resulted in significant reductions in nicotine self-administration (Fig. 1). The rats averaged 4.68 ± 0.90 nicotine infusions per session after control treatment. In comparisons, they averaged 3.82 ± 0.95 after 0.3 mg/kg, 2.90 ± 0.77 after 1 mg/kg and 3.12 ± 0.80 after 3 mg/kg of sazetidine-A. There were no differential sazetidine-A effects on nicotine self-administration in the first and second phases of dosing, with all of the doses being tested for the first and second time. The data presented in Fig. 1 are from the means of nicotine self-administration for each dose during the two test phases,

3.3. Sazetidine-A oral time effect function

Eighteen of the rats from the initial experiment continued with the second experiment. There was a significant ($F(1,17) = 7.62$, $p < 0.025$) main effect of sazetidine-A in reducing nicotine self-administration (Fig. 2). The interaction with interval between dosing and sazetidine-A treatment was not significant ($p = 0.58$). Fig. 3 shows the difference between nicotine infusions after the control vehicle administration and nicotine self-administration after the oral administration of 3 mg/kg sazetidine-A at different time points. There were no differential sazetidine-A effects on nicotine self-administration in the first and second phases of dosing, with all of the time intervals being tested for the first and second time. Thus, the results graphed are the means of

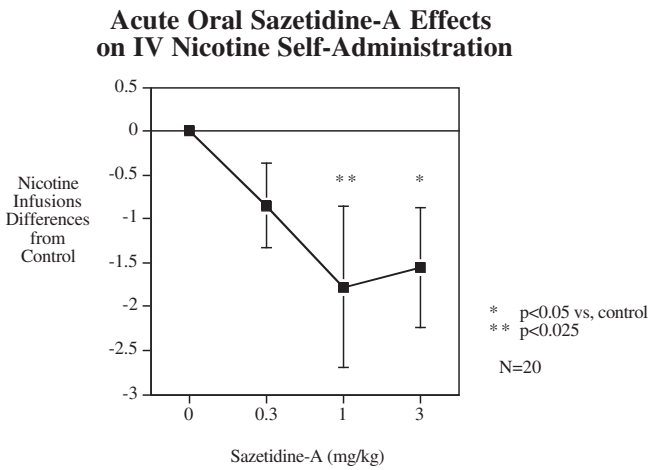

Fig. 1. Acute dose-effect (0.3–3 mg/kg) function of oral administration of sazetidine-A significantly reducing IV nicotine self-administration in female rats. The number of nicotine infusions per session different from vehicle control, average for each dose during both the first and second test phase (mean ± sem) (N = 20).

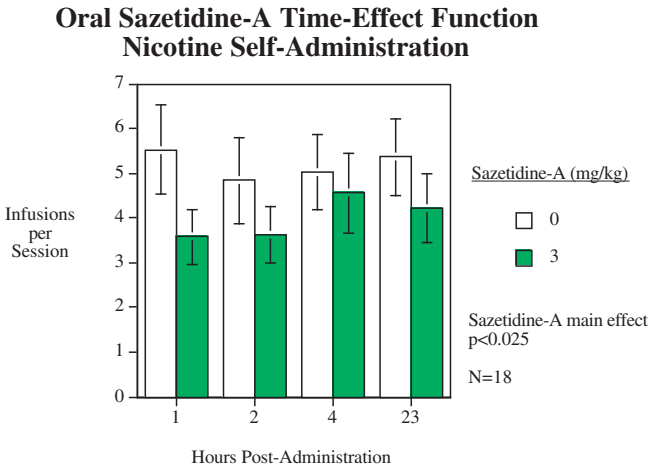

Fig. 2. Acute time-effect function for oral sazetidine-A (3 mg/kg) significantly (p < 0.025) reducing IV nicotine self-administration in female rats. The number of nicotine infusions per session average for each time interval for the two test phases (mean ± sem) (N = 18).

performance across the two repeated phases of testing.

4. Discussion

We previously found that acute subcutaneous (SC) injection or chronic SC infusions of sazetidine-A caused significant reductions in IV nicotine self-administration in rats (Johnson et al., 2012; Levin et al., 2010; Rezvani et al., 2010). Our current findings show that similar to systemic SC administration, oral administration of sazetidine-A is also effective in reducing nicotine intake. These findings support the role of α4β2 nicotinic receptors in nicotine addiction and advances the development of sazetidine-A as a possible aid for ~~as a~~ smoking cessation. A second important positive finding with regard to drug development is that after oral administration sazetidine-A maintains its efficacy over the course of a full day.

Prior research has shown that sazetidine-A significantly reverses attentional impairment caused by either muscarinic acetylcholine blockade with scopolamine or NMDA glutamate blockade with dizocilpine (MK-801) (Rezvani et al., 2011, 2012). Other studies have found anxiolytic and antidepressant effects of sazetidine-A (Caldarone et al., 2011; Turner et al., 2010) as well as analgesic effects (Cucchiaro et al., 2008). This demonstration that sazetidine-A is efficacious via the oral route of administration further supports the idea that it may be a useful oral medication that could be developed as a smoking cessation aid as well as for treatment of cognitive and/or emotional dysfunction. Sazetidine-A like nicotine and varenicline has been found in research to support a modest extent of self-administration, suggesting that it is not aversive to the rats (Paterson et al., 2010). They found that sazetidine-A self-administration is less robust than that seen for nicotine.

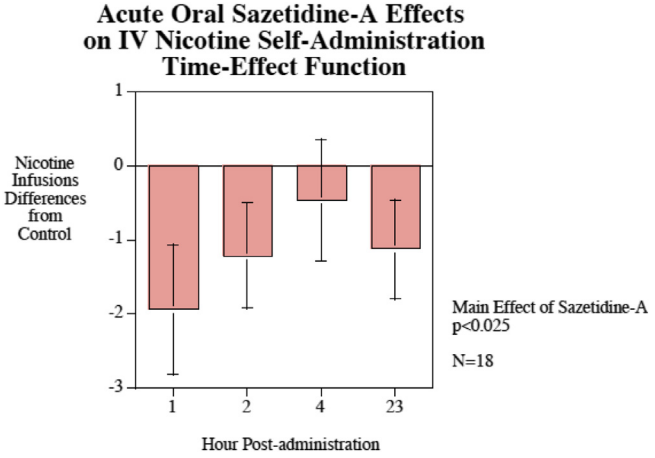

Fig. 3. Acute time-effect function for oral sazetidine-A (3 mg/kg) reducing IV nicotine self-administration. The number of nicotine infusions per session, difference from the control vehicle at each time point average for each time interval for the two test phases (mean ± sem) (N = 18).

Desensitization of α4β2 nicotinic receptors by sazetidine-A may be its mechanism of action to decrease nicotine self-administration. This can be related to the interaction of nicotinic receptors with the mesocorticolimbic dopamine (DA) system, which plays a key role in the rewarding effects of drugs of abuse (Nestler, 2005). Nicotine, similar to many other addictive drugs, increases the activity of dopaminergic neurons in the ventral tegmental area (VTA) and consequently at least initially elevates dopamine release in the nucleus accumbens (Balfour, 2015). One of the key nAChRs in the VTA controlling dopaminergic activity is an α4β2 nicotinic subtype, and sazetidine-A may exert its effects by desensitizing these particular nAChRs (Hussmann et al., 2012). This would render them resistant to subsequent stimulation by nicotine, resulting in inhibition or reduction of DA release in the nucleus accumbens.

It should also be considered that sazetidine-A, like nicotine itself, has both agonist and desensitizing effects on nAChRs (Katz and Thesleff, 1957; Langley and Dickenson, 1889). In addition to potently desensitizing α4β2 receptors, sazetidine-A has variable agonist activity at these receptors, depending on the receptor's subunit stoichiometry (Zwart et al., 2008). Thus, sazetidine-A appears to have nearly full agonist activity at $(\alpha4)_2(\beta2)_3$ receptors but nearly no agonist activity at $(\alpha4)_3(\beta2)_2$ receptors (Carbone et al., 2009). In either case, however, while any initial activation of α4β2 nAChRs by sazetidine-A would last only for seconds, its desensitization effect, which invariably follows the activation effect lasts more than an hour (Xiao et al., 2006). Thus, it is likely that the effects of sazetidine-A on nicotine self-administration are due to mainly to its prolonged desensitization of α4β2 nAChRs rather than the initial very brief activation of these receptors.

Further research is needed in the development of sazetidine-A for possible use as an aid for smoking cessation. In previous work we indexed the pharmacokinetics of sazetidine-A after subcutaneous administration (Hussmann et al., 2012). Sazetidine-A pharmacokinetics with oral administration remains to be done. Then the relationship of drug persistence in the brain to the persistence of behavioral effect can be determined,

In conclusion, our data showed that similar to systemic injection, oral sazetidine-A also significantly reduces IV nicotine self-administration in rats, and, more importantly, the effectiveness of sazetidine-A lasted for at 23–24 h. after the oral administration. The current findings that oral sazetidine-A has a long-lasting effect in reducing nicotine self-administration and based on our previous studies (Levin et al., 2010; Rezvani et al., 2010) support the contention that nAChR desensitization may be useful for the treatment of nicotine addiction and that sazetidine-A is a good candidate for further development leading to a possible clinical trial.

Acknowledgement

This research was supported by NIDA U19 grant DA027990.

Disclosure

Georgetown University holds a patent for Sazetidine-A. Drs. Y. Xiao and K.J. Kellar are its inventors.

References

Balfour, D.J., 2015. The role of mesoaccumbens dopamine in nicotine dependence. Curr. Top. Behav. Neurosci. 24, 55–98.

Caldarone, B.J., Wang, D., Paterson, N.E., Manzano, M., Fedolak, A., Cavino, K., Kwan, M., Hanania, T., Chellappan, S.K., Kozikowsk, i.A.P., Olivier, B., Picciotto, M.R., Ghavami, A., 2011. Dissociation between duration of action in the forced swim test in mice and nicotinic acetylcholine receptor occupancy with sazetidine, varenicline, and 5-I-A85380. Psychopharmacology 217, 199–210.

Carbone, A.L., Moroni, M., Groot-Kormelink, P.J., Bermudez, I., 2009. Pentameric concatenated (a4)2(b2)3 and (a4)3(b2)2 nicotinic acetylcholine receptors: subunit arrangement determines functional expression. Br. J. Pharmacol. 156, 970–981.

Cucchiaro, G., Xiao, Y., Gonzalez-Sulser, A., Kellar, K.J., 2008. Analgesic effects of sazetidine-A, a new nicotinic cholinergic drug. Anesthesiology 109 (3), 512–519.

Fiore, M.C., Smith, S.S., Jorenby, D.E., Baker, T.B., 1994. The effectiveness of the nicotine patch for smoking cessation - a meta-analysis. JAMA - Journal of the American Medical Association 271 (24), 1940–1947.

Freedman, R., 2007. Exacerbation of schizophrenia by varenicline. Am. J. Psychiatr. 164, 1269.

Hussmann, G.P., Turner, J.R., Lomazzo, E., Venkatesh, R., Cousins, V., Xiao, Y., Yasuda, R.P., Wolfe, B.B., Perry, D.C., Rezvani, A.H., Levin, E.D., Blendy, J.A., Kellar, K.J., 2012. Chronic sazetidine-A at behaviorally active doses does not increase nicotinic cholinergic receptors in rodent brain. J. Pharmacol. Exp. Ther. 343, 441–450.

Johnson, J.E., Slade, S., Wells, C., Petro, A., Sexton, H., Rezvani, A.H., Brown, M.L., Paige, M.A., McDowell, B.E., Xiao, Y., Kellar, K.J., Levin, E.D., 2012. Assessing the effects of chronic sazetidine-A delivery on nicotine self-administration in both male and female rats. Psychopharmacology 222, 269–276.

Jorenby, D.E., Hays, J.T., Rigotti, N.A., Azoulay, S., Watsky, E.J., Williams, K.E., Billing, C.B., Gong, J., Reeves, K.R., Group, V.P.S, 2006. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. JAMA - Journal of the American Medical Association 296, 56–63.

Katz, B., Thesleff, S., 1957. A study of the desensitization produced by acetylcholine at the motor end-plate. J. Physiol. Lond. 138, 63–80.

Langley, J.H., Dickenson, W.L., 1889. On the local paralysis of the peripheral ganglia and on the connexion of different classes of nerve fibres with them. Proceedings of the Royal Society 46, 423–431.

Levin, E.D., Westman, E.C., Stein, R.M., Carnahan, E., Sanchez, M., Herman, S., Behm, F.M., Rose, J.E., 1994. Nicotine skin patch treatment increases abstinence, decreases withdrawal symptoms and attenuates rewarding effects of smoking. J. Clin. Psychopharmacol. 14, 41–49.

Levin, E.D., Rezvani, A.H., Xiao, Y., Slade, S., Cauley, M., Wells, D., Hampton, D., Petro, A., Rose, J.E., Brown, M.L., Paige, M.A., McDowell, B.E., Kellar, K., 2010. Sazetidine-A, a selective α4β2 nicotinic receptor desensitizing agent and partial agonist reduces nicotine self-administration in rats. J. Pharmacol. Exp. Ther. 332, 933–939.

Morstad, A.E., Kutscher, E.C., Kennedy, W.K., Carnahan, R.M., 2008. Hypomania with agitation associated with varenicline use in bipolar II disorder. Ann. Pharmacother. 42, 288.

Nestler, E.J., 2005. Is there a common molecular pathway for addiction? Nat. Neurosci. 8, 1445–1449.

Paterson, N.E., Min, W., Hackett, A., Lowe, D., Hanania, T., Caldarone, B., Ghavami, A., 2010. The high-affinity nAChR partial agonists varenicline and sazetidine-A exhibit reinforcing properties in rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 1455–1464.

Quick, M., Lester, R., 2002. Desensitization of neuronal nicotinic receptors. J. Neurobiol. 53, 457–478.

Rezvani, A.H., Slade, S., Wells, C., Petro, A., Li, T.K., Lumeng, L., Xiao, Y., Brown, M.L., Paige, M.A., McDowell, B.E., Kellar, K.J., Rose, J.E., Levin, E.D., 2010. Sazetidine-A, a selective α4β2 nicotinic acetylcholine receptor desensitizing agent and partial agonist reduces both alcohol and nicotine self-administration in selectively-bred alcohol preferring (P) rats. Psychopharmacology 211, 161–174.

Rezvani, A.H., Cauley, M., Sexton, H., Xiao, Y., Brown, M.L., Paige, M.A., McDowell, B.E., Kellar, K.J., Levin, E.D., 2011. Sazetidine-A, a selective alpha4beta2 nicotinic acetylcholine receptor ligand: effects on dizocilpine and scopolamine-induced attentional impairments in female Sprague-Dawley rats. Psychopharmacology 215, 621–630.

Rezvani, A.H., Cauley, M., Xiao, Y., Kellar, K.J., Levin, E.D., 2012. Effects of chronic sazetidine-A, a selective β2* nicotinic receptor desensitizing agent on pharmacologically-induced impaired sustained attention in rats. Psychopharmacology 222, 269–276.

Rollema, H., Chambers, L.K., Coe, J.W., Glowa, J., Hurst, R.S., Lebel, L.A., Lu, Y., Mansbach, R.S., Mather, R.J., Rovetti, C.C., Sands, S.B., Schaeffer, E., Schulz, D.W., Tingley 3rd, F.D., Williams, K.E., 2007. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology 52 (3), 985–994.

Turner, J.R., Castellano, L.M., Blendy, J.A., 2010. Nicotinic partial agonists varenicline and sazetidine-A have differential effects on affective behavior. J. Pharmacol. Exp. Ther. 334 (2), 665–672.

Xiao, Y., Fan, H., Musachio, J.L., Wei, Z.L., Chellappan, S.K., Kozikowski, A.P., Kellar, K.J., 2006. Sazetidine-A, a novel ligand that desensitizes alpha4beta2 nicotinic acetylcholine receptors without activating them. Mol. Pharmacol. 70, 1454–1460.

Zwart, R., Carbone, A.L., Moroni, M., Bermudez, I., Mogg, A.J., Folly, E.A., Broad, L.M., Williams, A.C., Zhang, D., Ding, C., Heinz, B.A., Sher, E., 2008. Sazetidine-A is a potent and selective agonist at native and recombinant alpha 4 beta 2 nicotinic acetylcholine receptors. Mol. Pharmacol. 73, 1838–1843.