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ASSESSING THE EFFECTS OF CHRONIC SAZETIDINE-A DELIVERY ON NICOTINE SELF-ADMINISTRATION IN BOTH MALE AND FEMALE RATS

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Abstract

Rationale—Sazetidine-A is a selective α4β2 nicotinic receptor desensitizing agent and partial agonist. It has been shown in previous studies to significantly reduce nicotine self-administration in rats after acute or repeated injections. However, the effects of continuous chronic infusions of sazetidine-A on maintenance of nicotine self-administration and relapse after abstinence have yet to be examined.

Objectives—This study evaluated the efficacy of continuous sazetidine-A infusions (sc) over a period of four weeks to reduce nicotine self-administration in male and female Sprague-Dawley rats.

Methods—Sazetidine-A was administered via Alzet osmotic minipumps to young adult female and male rats at doses of 0, 2 or 6 mg/kg/day for four weeks. The effects of sazetidine-A on IV nicotine self-administration were examined in repeated 3-hour sessions over the first two weeks of infusion followed by one week of forced abstinence from nicotine and one week of resumed nicotine access.

Results—The 6 mg/kg/day sazetidine-A dose significantly reduced overall nicotine self-administration compared with vehicle control across the sessions for both male ($p<0.001$) and female ($p<0.05$) rats. The lower 2 mg/kg/day sazetidine-A infusion dose was effective in reducing nicotine self-administration for male ($p<0.001$), but not female rats. No attenuation in sazetidine-A effectiveness was seen over the course of the four-week treatment. In the vehicle control group, male rats self-administered significantly ($p<0.001$) more nicotine than females.

Conclusions—The continuing effectiveness of sazetidine-A in reducing nicotine self-administration in both male and female rats supports its promise as a new treatment to help people successfully quit smoking.

Keywords

Nicotine; Sazetidine-A; chronic; Self-administration; Sex differences

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Introduction

Nicotine addiction remains a major public health problem. Currently approved tobacco/smoking cessation treatments including various types of nicotine replacement, buproprion 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, and new more effective treatments are needed. In addition, a greater diversity of effective treatments will help with the tailoring of the most effective therapy to the needs of the diverse smoker population. Many of the available treatments focus on nicotine's action at neuronal nicotinic acetylcholine receptors (nAChRs). Nicotine both activates and desensitizes nAChRs (Katz and Thesleff 1957; Quick and Lester 2002), but the relative roles of nAChR activation and desensitization for the pharmacological effects of nicotine are still unclear. Better understanding the relative roles of nAChR activation and desensitization in nicotine reinforcement and self-administration should help in the development of more effective treatments to aid smoking cessation.

Current treatments that focus on nAChRs have been successful in reducing nicotine self-administration in the short-term, but have failed to produce long-term effects for the majority of smokers. One such pharmacological treatment is varenicline (Rollema et al. 2007). Varenicline, a partial α4β2 agonist, has been shown to reduce smoking relative to placebo and bupropion (Jorenby et al. 2006), but it has several potential drawbacks. For example, there is a relatively high rate of adverse side effects associated with varenicline, including nausea, abnormal dreams, and more rarely exacerbation of psychiatric symptoms (Freedman 2007; Morstad et al. 2008). These side effects may be due to actions at α3β4* and/or α7 nAChRs, rather than the α4β2 subtype. A drug that more selectively affects the α4β2 receptor may be less likely to produce the adverse side effects associated with varenicline. One possibility is the more α4β2-selective drug sazetidine-A.

Sazetidine-A is a recently developed nAChR partial agonist with a high affinity and selectivity for α4β2 receptors. This compound potently desensitizes α4β2 receptors and has a long lasting effect in cells (Xiao et al. 2006). Similar to other compounds that act at the α4β2 receptors, such as varenicline (Jorenby et al. 2006), sazetidine-A has been found to be effective in reducing nicotine self-administration after both acute and repeated administration at 3 mg/kg, with only modest effects on locomotion and food-motivated responding (Levin et al. 2010; Rezvani et al. 2010). However, the effectiveness of a chronic long-term, continuous infusion has yet to be evaluated.

Moreover, important sex differences have been found in nicotine self-administration by rats, as well as smoking cessation by people. Therefore, we believed it to be important to consider sex differences when examining both nicotine self-administration, and when evaluating the effectiveness of a compound to reduce nicotine self-administration. Sex differences in a rodent model of nicotine self-administration have previously been explored (Chaudhri et al. 2005; Donny et al. 2000; Rezvani et al. 2008). In the earlier study, Donny et al (2000) reported that female rats acquired nicotine self-administration more rapidly than male rats, and had a higher break point on a progressive ratio (PR) schedule. However, whether this sex difference was due to differential sensitivity to nicotine or the presentation of a visual stimulus was initially unclear. It was later confirmed that female rats both acquired nicotine self-administration quicker and responded more on the active lever both in the presence and absence of the visual stimulus (Chaudhri et al. 2005). Females also self-administered more than males in a study where animals had 6-OHDA lesions (Rezvani et al. 2008). In a rodent nicotine self-administration model, females consistently responded more than males for nicotine, suggesting that males and females might also respond differentially to similar pharmacological treatments. Acute sazetidine-A, has been found to significantly

reduce nicotine self-administration in both male and female rats (Levin et al. 2010; Rezvani et al. 2010). Repeated sazetidine-A injections significantly reduce nicotine self-administration in female rats but male rats remain to be evaluated (Levin et al. 2010; Rezvani et al. 2010).

The aim of the present study was to evaluate the effectiveness of four weeks of continuous infusion of sazetidine-A in reducing nicotine self-administration in both male and female Sprague-Dawley rats. With the constant infusion, we could distinguish the effects of the sazetidine-A across the course of 3-hour nicotine self-administration sessions without the varying pharmacokinetics seen after an acute injection. The persistence of the sazetidine-A effects was measured for two weeks of nicotine access as well as an additional week of resumed access after a one-week period of enforced abstinence. It was hypothesized that sazetidine-A would effectively reduce nicotine self-administration in both males in females for the entire period of treatment with differential sensitivity between the sexes.

Methods

Subjects

Young adult male (n=30) and female (n=26) Sprague-Dawley rats (Taconic Lab, Germantown, NY, USA) were used in the present study. 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 so that experimental sessions occurred during the active part 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 minutes after the completion of their experimental session and were maintained at roughly 85% of free-feeding weight. Males were fed 21–23g of feed daily and their weights ranged from 180–300g (start to end of study). Females were fed 17–19g of feed daily. Their weights ranged from 150–250g (start to end of study). Animals progressively and healthily gained weight throughout the study. At the end of the study catheter patency tests were conducted and two animals with an obstructed catheter were dropped from the study.

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 via NIDA. Sazetidine-A was delivered sc continuously for four weeks via osmotic minipumps (Alzet model 2ML4, Durect Inc., Cupertino, CA, USA) at one of three doses: 0, 2 or 6 mg/kg/day. Sazetidine-A was delivered for the first two weeks with daily access to nicotine self-administration sessions, followed by one week of enforced abstinence from nicotine, and one week of resumption of nicotine access.

Nicotine bitartrate solutions were prepared weekly in sterilized isotonic saline. The dose used for self-administration (0.03 mg/kg/infusion) was calculated as a function of the nicotine free base 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) for sterilization. Between sessions all nicotine was kept in a dark refrigerator.

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 a mixture of ketamine (60 mg/kg) and dormitor (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 hours to recover from surgery before experiencing nicotine self-administration sessions.

Following surgery animals experienced 5 experimental sessions 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-minute period where the cue lights went out, the house light came on and correct responses were recorded but not reinforced. After the initial 5 sessions of nicotine self-administration the osmotic minipumps containing either saline or one of two doses of sazetidine-A (2, or 6 mg/kg/day) were implanted under ketamine anesthesia and animals then experienced 2 weeks of nicotine self-administration sessions followed by one week of enforced abstinence during which the rats were not tested, and one week of resumption of nicotine access. Each session lasted for 3 hours with 5 sessions occurring per week (Monday-Friday).

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

Data Analysis

The data were evaluated with a mixed between and within subject design analysis of variance (ANOVA). Sazetidine-A dose and sex were between-subjects factors. Weeks of testing and 15-min time blocks within session 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. Dunnett's test was used to compare each of the sazetidine-A dose groups to control.

Results

Pretraining

During the three pellet training sessions there was a significant effect of sex $F(1,54)=42.41$, $p<0.0005$) with males earning a mean of 123.6±4.7 pellets per session and females earning 82.9±4.0. During the five sessions of initial training for nicotine self-administration there was a significant main effect of session ($F(4,216)=5.25$, $p<0.005$). The mean number of nicotine infusions per session rose from 10.1±0.7 during the first session to a peak of 16.4±0.8 during the third session to 12.5±0.7 during the fifth session. There was also a significant main effect of block within session ($F(11,594)=21.99$, $p<0.0005$). The main effect of sex was not significant. There was a significant sex x 15-minute time block within session interaction ($F(11,594)=1.86$, $p<0.05$). Follow-up trend analyses over the time blocks for each sex showed that for males only the linear ($p<0.0005$) and quadratic ($p<0.0005$) trends were significant. For females the linear ($p<0.0005$), quadratic ($p<0.0005$), cubic ($p<0.0005$), quartic ($p<0.025$) trends were significant. As can be seen in figure 1, the nicotine self-administration for the females showed rapid decline after the initial burst in responding for nicotine followed by a significant undulating pattern with discrete periods of

increasing and decreasing nicotine self-administration. In contrast, the males showed a far simpler pattern of initial high responding with a smoother decline in responding.

Sazetidine-A Effects

Sazetidine-A significantly ($F(2,49)=8.27$, $p<0.001$) decreased nicotine self-administration with no appearance of diminished effectiveness across two weeks of continued nicotine access and during the resumption week after enforced abstinence, as well (Fig. 2). The main effect of sex was not significant, but there was a significant sazetidine-A x sex interaction ($F(2,49)=3.38$, $p<0.05$). Tests of the simple main effects of sazetidine-A within each sex showed that for males, both the 2 mg/kg/day ($p<0.005$) and 6 mg/kg/day ($p<0.005$) significantly reduced nicotine self-administration, while females experienced significant reduction ($p<0.05$) at the 6 mg/kg/day dose only (Fig. 2). There was also a significant main effect of block within session ($F(11,539)=16.76$, $p=0.0001$). There was no apparent decrease of effectiveness of sazetidine-A over the course of weeks (Fig. 3A) in either males or females (Fig. 3B). During the course of the 3-hour test session the sazetidine-A, effects were most apparent after the initial burst of responding (Fig. 4). The sazetidine-A treated rats settled into a lower asymptotic level of nicotine self-administration after the initial flurry of self-administration during the first 15-min block. This pattern was consistent across weeks.

Discussion

Chronic sazetidine-A sc infusions over a course of four weeks significantly decreased nicotine self-administration in both male and female rats with no indication of a lessening of effect with continuing treatment, but appeared to be more potent in males than females. During the course of the three-hour self-administration sessions, sazetidine-A had its most prominent effect in reducing the asymptotic levels of nicotine self-administration rather than the initial burst of responding at the beginning of each session. Sazetidine-A induced reduction of nicotine self-administration was more prominent in males probably because under control conditions male rats self-administered more nicotine than females. The sazetidine-A treatment eliminated this sex-difference.

In previous studies, we have shown that acute sazetidine-A treatment significantly reduced nicotine self-administration in male and female rats (Levin et al. 2010; Rezvani et al. 2010). Continued efficacy was seen with repeated injections over two weeks. The effective injection dose did not significantly alter locomotor activity over a one-hour session, though there was some reduction in activity during the initial part of the session. In addition, recent unpublished data in our laboratory has shown that sazetidine-A, at 6 mg/kg delivered via osmotic pump, decreased response latency and errors of omission on an attention task. These unpublished data support the interpretation that sedative effects at the dose range evaluated were not responsible for the decrease in nicotine self-administration. While we did find that acute sazetidine-A did significantly reduce responding for food pellets, the sazetidine-A effect on food administration (83% of baseline) was significantly and consistently less than the effect on nicotine administration (43% of baseline). Alternatively, animals that received sazetidine-A self-administered nicotine 60% less than control animals and administered food 28% less than control animals. This supports a greater effect of sazetidine-A on nicotine maintained responding.

Furthering this line of research the current study showed that continuous infusions of sazetidine-A over four weeks significantly reduced nicotine self-administration in male and female rats, with no sign of diminished response over that period. The lower sazetidine-A dose (2 mg/kg/day) lowered nicotine self-administration by 41% during the first week of treatment, 45% during the second week and 38% during the fourth week of treatment after one week of enforced abstinence. The higher sazetidine-A dose (6 mg/kg/day) reduced

nicotine self-administration by 51% during the first week, 53% during the second week and 55% during the fourth week of treatment after one week of enforced abstinence.

Use of continuous infusion allowed the discrimination of effectiveness for sazetidine-A over the course of the three-hour test sessions without the confound of shifting drug levels over that time as with acute or repeated injections. As shown in figure 4, the principal effect of sazetidine-A was in reducing the continuing baseline of nicotine self-administration during middle and later portions of the sessions rather than during the burst of nicotine self-administration during the initial 15-minute interval of a session. This was true throughout nicotine access: the first two weeks of therapy, as well as during the week of resumed access (week four of therapy) after a week of enforced abstinence. This could have implications for treatment. While sazetidine may not affect the initial loading phase, the present study suggests sazetidine-A could effectively reduce smoking over the course of a day and may provide efficacy in reducing the risk of slips turning into relapses. Whether this is the case will need to be determined in further research.

Varenicline was found to also reduce alcohol consumption and seeking in rats (Steensland et al. 2007) and even in humans (McKee et al. 2009). Similarly, sazetidine-A was also shown to reduce alcohol intake after acute and repeated administrations (Rezvani et al. 2010). In that study alcohol-preferring rats experienced significantly reduced alcohol consumption, even after alcohol post-deprivation when alcohol consumption is usually increased. Similar to the present study sazetidine-A administration also reduced nicotine self-administration in previous studies (Levin et al. 2010; Rezvani et al. 2010). These findings suggest that nicotine and alcohol share similar neuronal mechanisms and desensitizing α4β2 nicotinic receptors with chronic sazetidine-A might be an effective treatment in reducing both nicotine and alcohol intake.

Male rats in the vehicle condition earned significantly more nicotine infusions on average than female rats over the course of the first two weeks and the resumption week. In contrast, several studies have shown female rats earning more nicotine infusions than male rats (Chaudhri et al. 2005; Donny et al. 2000; Lynch 2009; Rezvani et al., 2008) However, the sex difference observed in the vehicle condition was not present during the five sessions of initial training for nicotine self-administration. It is unlikely that deprivation levels played a role in this sex difference as males and females were fed sex-appropriate amounts to maintain approximately 85% of free-feeding weight. It is possible that the differences that emerged after the osmotic pump implantation were merely due to sex differences in the reaction or recovery from an additional surgery. Another possibility is that males and females achieved differing plasma concentrations of sazetidine-A. Of course this was unlikely an issue for the present study as the sex differences emerged during the vehicle condition, but it could be an important variable to consider for subsequent studies.

Despite this sex difference in vehicle responding, sazetidine-A was still effective in reducing nicotine self-administration in both sexes. Further, the two sexes can be viewed as two different groups of smokers. While these two groups had different baseline levels of nicotine self-administration, sazetidine-A was effective in reducing nicotine self-administration for both groups with disparate levels of baseline nicotine self-administration. This result suggests some evidence of the generality of sazetidine-A's ability to reduce nicotine self-administration across a wide range of nicotine consumers.

Sazetidine-A remains a candidate for treating nicotine dependence. Sazetidine-A was effective in reducing nicotine self-administration for both males and females, which each represented different levels of nicotine self-administration before sazetidine-A treatment. However, additional compounds that selectively desensitize α4β2 nicotinic receptors should

be tested to further prove the utility and specificity of drugs with this mechanism of action in reducing nicotine self-administration. Future studies should investigate the specificity of the effect further. Questions addressing whether the agonist action or desensitizing action play a more critical role, or is the combination of these two effects is important should be explored

In summary, treatment based on desensitization of α4β2 nicotinic receptors such as is induced by sazetidine-A may be useful for promoting cessation of tobacco smoking and should be investigated further for this possible use.

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References

Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib MA, Craven LA, Allen SS, Sved AF, Perkins KA. Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. Psychopharmacology (Berl). 2005; 180:258–266. [PubMed: 15682294]

Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldovan V, Booth S, Mielke MM, Hoffman A, McCallum S. Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. Psychopharmacology (Berl). 2000; 151:392–405. [PubMed: 11026746]

Fiore MC, Smith SS, Jorenby DE, Baker TB. The effectiveness of the nicotine patch for smoking cessation. A meta-analysis. JAMA. 1994; 271:1940–1947. [PubMed: 8201739]

Freedman R. Exacerbation of schizophrenia by varenicline. Am J Psychiatry. 2007; 164:1269. [PubMed: 17671295]

Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. JAMA. 2006; 296:56–63. [PubMed: 16820547]

Katz B, Thesleff S. A study of the desensitization produced by acetylcholine at the motor end-plate. J Physiol. 1957; 138:63–80. [PubMed: 13463799]

Levin ED, Rezvani AH, Xiao Y, Slade S, Cauley M, Wells C, Hampton D, Petro A, Rose JE, Brown ML, Paige MA, McDowell BE, Kellar KJ. Sazetidine-A, a selective alpha4beta2 nicotinic receptor desensitizing agent and partial agonist, reduces nicotine self-administration in rats. J Pharmacol Exp Ther. 2010; 332:933–939. [PubMed: 20007754]

Levin ED, Westman EC, Stein RM, Carnahan E, Sanchez M, Herman S, Behm FM, Rose JE. Nicotine skin patch treatment increases abstinence, decreases withdrawal symptoms, and attenuates rewarding effects of smoking. J Clin Psychopharmacol. 1994; 14:41–49. [PubMed: 8151002]

Lynch WJ. Sex and ovarian hormones influence vulnerability and motivation for nicotine during adolescence in rats. Pharmacol Biochem Behav. 2009; 94:43–50. [PubMed: 19619575]

McKee SA, Harrison EL, O'Malley SS, Krishnan-Sarin S, Shi J, Tetrault JM, Picciotto MR, Petrakis IL, Estevez N, Balchunas E. Varenicline reduces alcohol self-administration in heavy-drinking smokers. Biol Psychiatry. 2009; 66:185–190. [PubMed: 19249750]

Morstad AE, Kutscher EC, Kennedy WK, Carnahan RM. Hypomania with agitation associated with varenicline use in bipolar II disorder. Ann Pharmacother. 2008; 42:288–289. [PubMed: 18198241]

Quick MW, Lester RA. Desensitization of neuronal nicotinic receptors. J Neurobiol. 2002; 53:457–478. [PubMed: 12436413]

Rezvani AH, Eddins D, Slade S, Hampton DS, Christopher NC, Petro A, Horton K, Johnson M, Levin ED. Neonatal 6-hydroxydopamine lesions of the frontal cortex in rats: persisting effects on locomotor activity, learning and nicotine self-administration. Neuroscience. 2008; 154:885–897. [PubMed: 18511204]

Rezvani AH, Slade S, Wells C, Petro A, Lumeng L, Li TK, Xiao Y, Brown ML, Paige MA, McDowell BE, Rose JE, Kellar KJ, Levin ED. Effects of sazetidine-A, a selective alpha4beta2 nicotinic acetylcholine receptor desensitizing agent on alcohol and nicotine self-administration in selectively bred alcohol-preferring (P) rats. Psychopharmacology (Berl). 2010; 211:161–174. [PubMed: 20535453]

Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, Sands SB, Schaeffer E, Schulz DW, Tingley FD 3rd, Williams KE. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology. 2007; 52:985–994. [PubMed: 17157884]

Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. Proc Natl Acad Sci U S A. 2007; 104:12518–12523. [PubMed: 17626178]

Xiao Y, Fan H, Musachio JL, Wei ZL, Chellappan SK, Kozikowski AP, Kellar KJ. Sazetidine-A, a novel ligand that desensitizes alpha4beta2 nicotinic acetylcholine receptors without activating them. Mol Pharmacol. 2006; 70:1454–1460. [PubMed: 16857741]

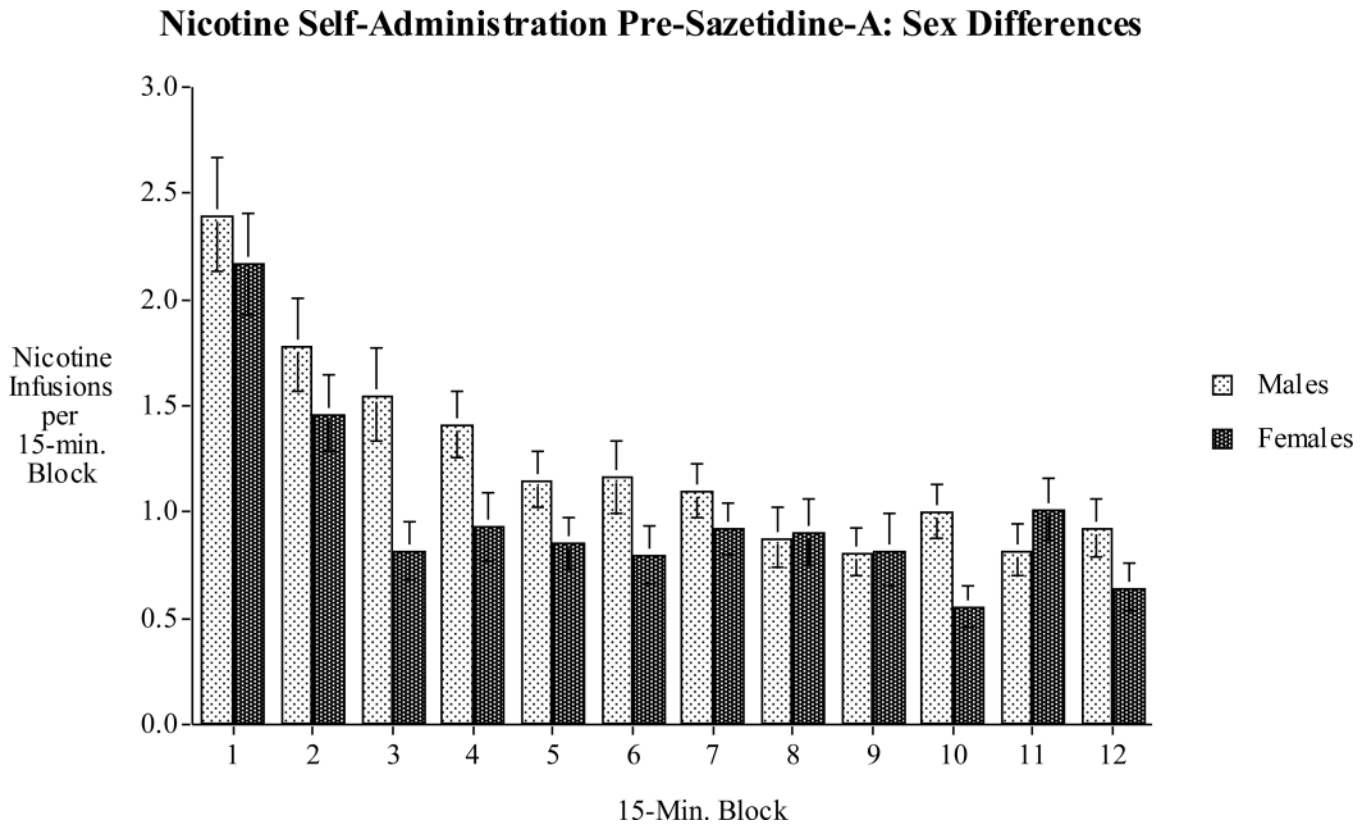


Figure 1.

Pretraining nicotine self-administration (mean of sessions 1–5±sem) of male (N=30) and female (N=26) rats during each of the 12 15-min blocks within the 3-hour session. Main effect of 15-min time block ($p<0.0005$). Sex x Block interaction ($p<0.05$). Significant trends over time blocks for each sex: Male Linear ($p<0.0005$) and Quadratic ($p<0.0005$); Female Linear ($p<0.0005$), Quadratic ($p<0.0005$), Cubic ($p<0.0005$) and Quartic ($p<0.05$)

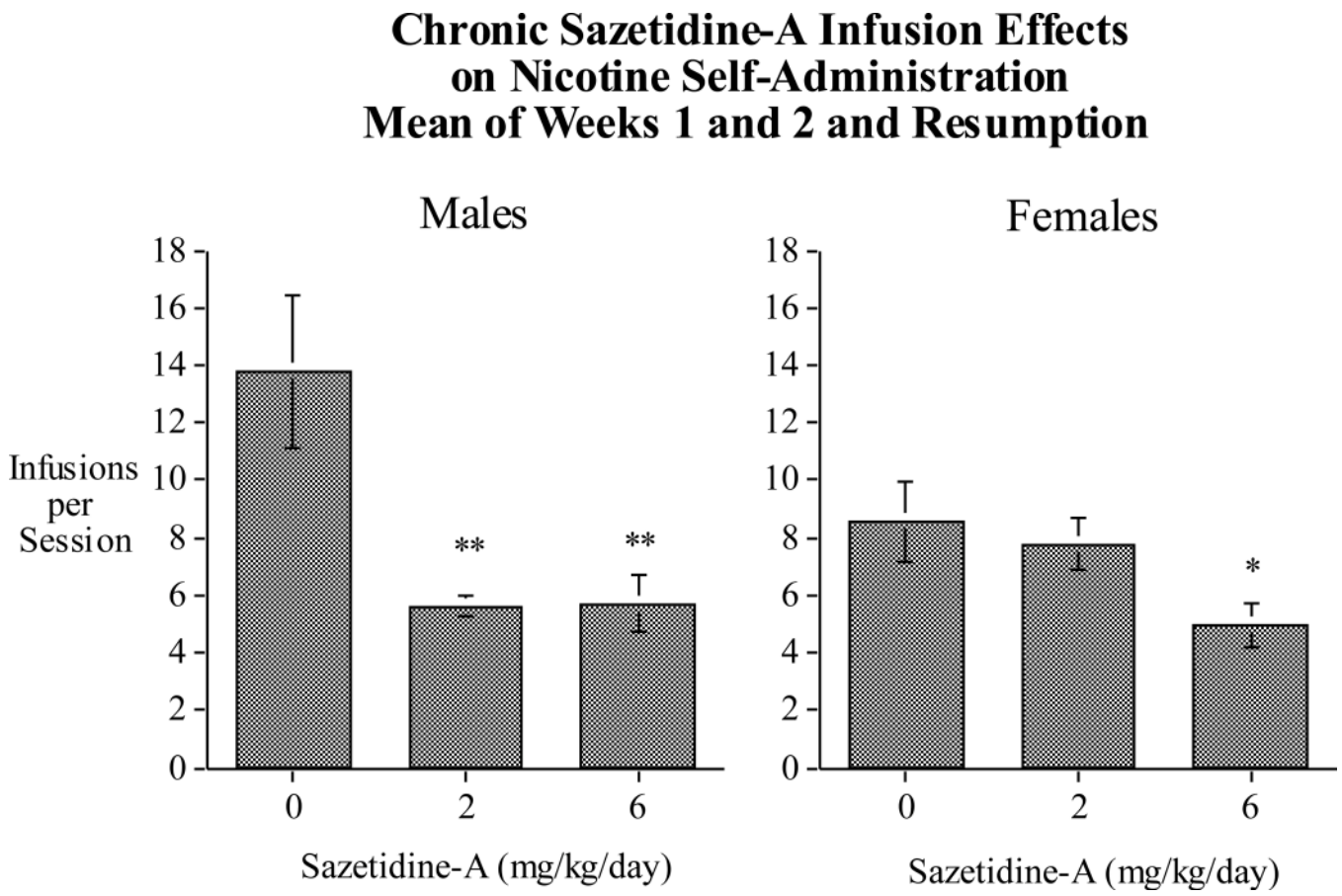
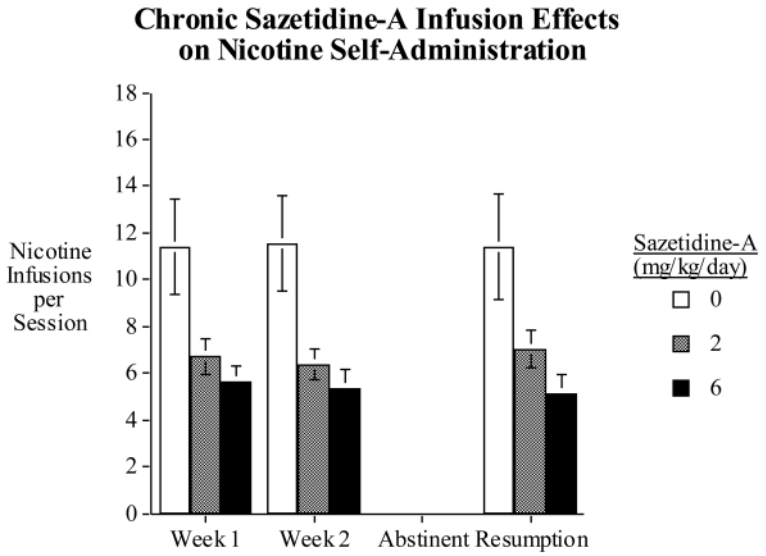


Figure 2.
Chronic sazetidine-A effects on nicotine self-administration mean for all weeks Control vs. Sazetidine-A treated * $p<0.025$, ** $p<0.005$, (mean±sem)

A



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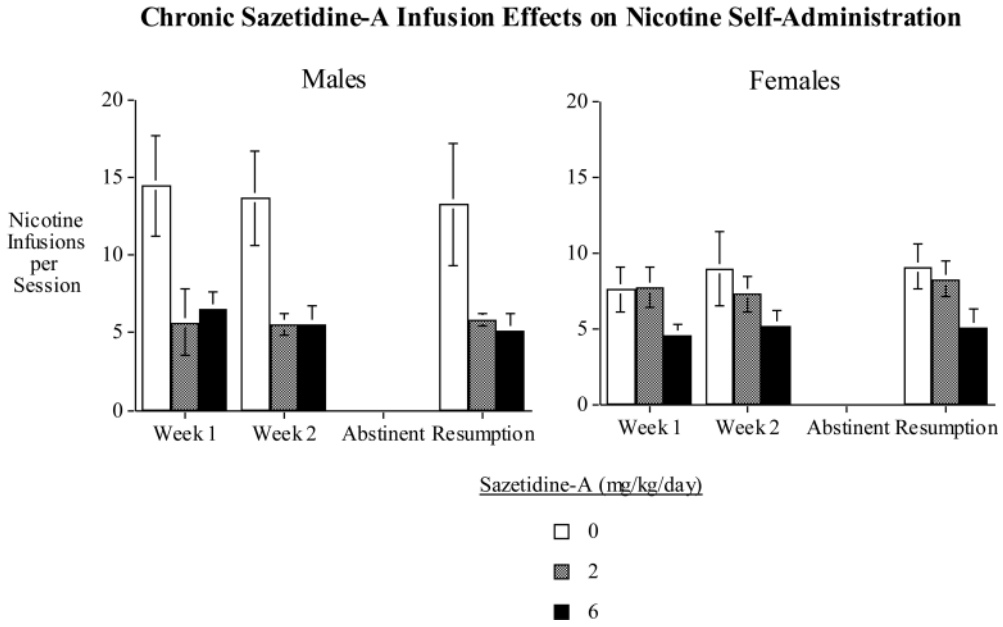

Figure 3.
Chronic sazetidine-A effects on nicotine self-administration for each week A) Both sexes, B) Each sex (mean±sem)

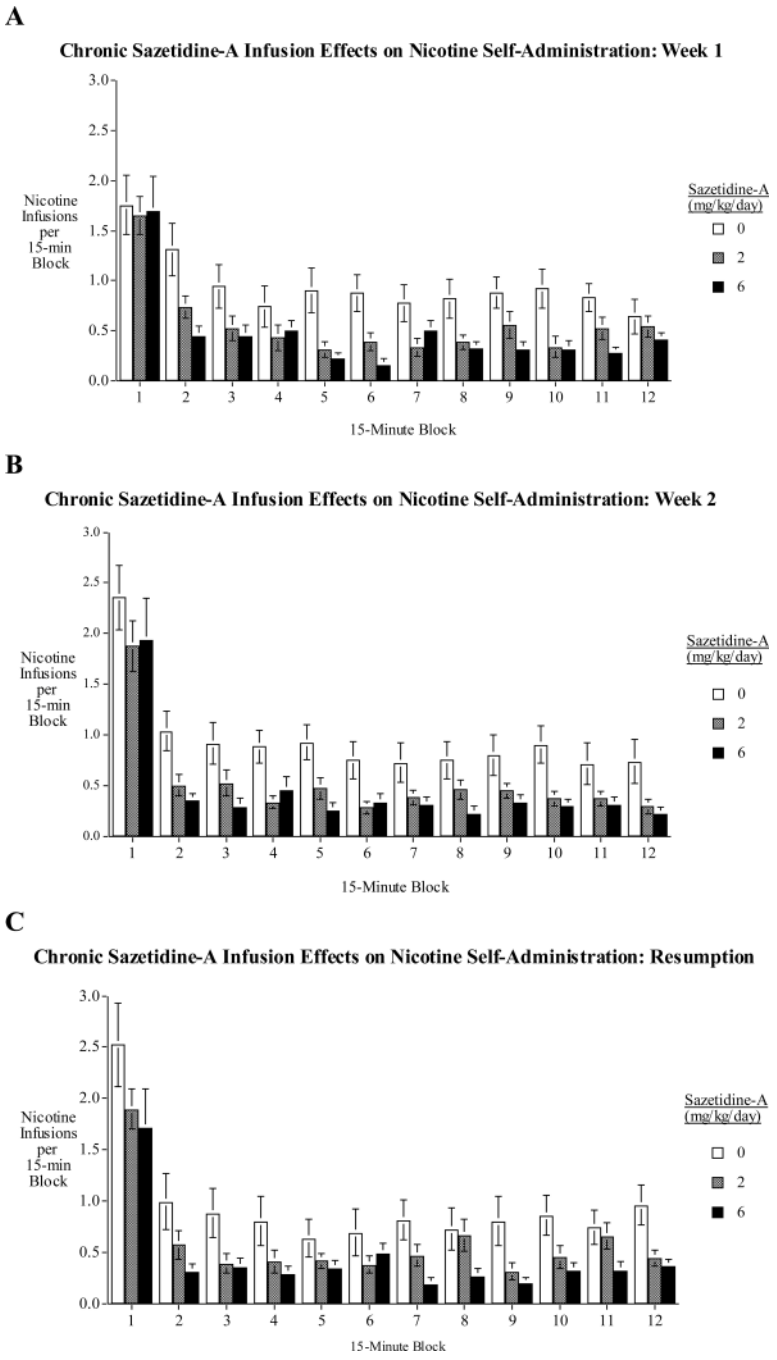

Figure 4.

Chronic sazetidine-A effects on nicotine self-administration during 15-min blocks during the 3-hr session for both sexes A) Week 1 Sazetidine-A main effect ($p<0.01$), Control vs 2 mg/kg/day ($p<0.01$), Control vs 6 mg/kg/day ($p<0.01$),, B) Week 2 Sazetidine-A main effect ($p<0.005$), Control vs 2 mg/kg/day ($p<0.01$), Control vs 6 mg/kg/day ($p<0.01$), C) Resumption Week Sazetidine-A main effect ($p<0.025$), Control vs 2 mg/kg/day ($p<0.05$), Control vs 6 mg/kg/day ($p<0.01$) (mean±sem)