

Published in final edited form as:

Eur J Pharmacol. 2011 March 25; 655(1-3): 52–58. doi:10.1016/j.ejphar.2011.01.009.

Both GABA_B receptor activation and blockade exacerbated anhedonic aspects of nicotine withdrawal in rats

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Abstract

Nicotine dependence is maintained by the aversive, depression-like effects of nicotine withdrawal and the rewarding effects of acute nicotine. GABA_B receptor antagonists exhibit antidepressant-like effects in rodents, whereas GABA_B receptor agonists attenuate the rewarding effects of nicotine. Recent studies with GABA_B receptor positive modulators showed that these compounds represent potentially improved medications for the treatment of nicotine dependence because of fewer side-effects than GABA_B receptor agonists. Thus, GABA_B receptor agonists and antagonists, and GABA_B receptor positive modulators may have efficacy as smoking cessation aids by targeting different aspects of nicotine dependence and withdrawal. The present study assessed the effects of the GABA_B receptor agonist CGP44532, the GABA_B receptor antagonist CGP56433A, and the GABA_B receptor positive modulator BHF177 on the anhedonic aspects of nicotine withdrawal. Rats were prepared with stimulating electrodes in the posterior lateral hypothalamus. After establishing stable intracranial self-stimulation (ICSS) thresholds, rats were prepared with subcutaneous osmotic minipumps delivering either nicotine or saline for 7 or 14 days. ICSS thresholds were assessed 6 h post-pump removal. Thirty hours after pump removal, CGP44532, CGP56433A, and BHF177 were administered 30 min prior to ICSS testing. Both GABA_B receptor activation (CGP44532 and BHF177) and blockade (CGP56433A) elevated ICSS thresholds in all groups, resulting in exacerbated effects of nicotine withdrawal in the nicotine-treated groups. These similar effects of GABA_B receptor activation and blockade on the anhedonic depression-like aspects of nicotine withdrawal were surprising and perhaps reflect differential efficacy of these compounds at presynaptic hetero- and autoreceptors, as well as postsynaptic, GABA_B receptors.

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Conflict of interest: The authors declare no conflicts of interest.

Keywords

GABA; positive modulators; CGP44532; CGP56433A; BHF177; depression-like state; ICSS; brain stimulation reward

1. Introduction

γ -aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, acting through ionotropic GABA_A and GABA_C, and metabotropic GABA_B receptors. GABA_B receptors are crucial in neurotransmission (Bowery et al., 2002), the pathophysiology of depression (Cryan and Slattery, 2010), and the modulation of reward processes (for review, Vlachou and Markou, 2010). Thus, GABA_B receptors are a promising target for the treatment of drug, including nicotine, dependence that is characterized by alterations in reward processes [(for reviews, Buchhalter et al., 2008; Cryan et al., 2003b; Vlachou and Markou, 2010)].

Findings supporting a role of GABA transmission in nicotine dependence include observations that acute nicotine administration modulates inhibitory GABAergic and excitatory glutamatergic inputs to ventral tegmental area (VTA) dopaminergic neurons (Mansvelter et al., 2002). The VTA to the nucleus accumbens dopaminergic projection is a critical component of the neurocircuit involved in drug dependence (Di Chiara and Imperato, 1988). Thus, chronic nicotine exposure could induce neuroadaptations in GABA_B receptor number/function, in addition to changes in other neurotransmitter systems/receptors, that could contribute to the depression-like aspects of nicotine withdrawal. The depression-like aspects of nicotine withdrawal are an important motivational factor in the perpetuation of the harmful tobacco smoking habit in humans (Hughes, 2007; Kenny and Markou, 2001).

Emerging, but contradictory, evidence from clinical and preclinical studies implicates changes in GABAergic function in the pathophysiology of non-drug-induced depression. Specifically, acute administration of GABA_B receptor antagonists exhibited antidepressant-like effects in the forced swim test in rats (Frankowska et al., 2007; Slattery et al., 2005), mice and olfactory bulbectomized rats (Nowak et al., 2006). Further, repeated administration of a GABA_B receptor antagonist attenuated decreases in sucrose consumption, a measure of hedonia, seen after exposure to chronic mild stress (Nowak et al., 2006), and improved learned helplessness in rats [(Nakagawa et al., 1999); but see (Sufka et al., 2009)]. Consistent with these findings, GABA_{B1} knockout mice displayed antidepressant-like behavior (Jacobson et al., 2007a; Jacobson and Cryan, 2005; Mombereau et al., 2004, 2005). Thus, blockade or loss of GABA_B receptor function induces an antidepressant-like phenotype in rodents.

In contrast to rodent studies, clinical studies showed decreased GABA levels in plasma, cerebrospinal fluid, and cortex in depressed individuals [(Sanacora et al., 2004, 2006); for review, (Sanacora and Saricicek, 2007)], suggesting that GABA_B receptor agonists or positive modulators, rather than antagonists, may have antidepressant properties [(Esel et al., 2008; Pilc and Nowak, 2005); but see (Nakagawa et al., 1996a,b,c; Slattery et al., 2005)]. In contradiction to the above clinical studies, GABA_B receptor agonists and positive modulators exhibited antidepressant-like effects in the forced swim test in rats (Frankowska et al., 2007).

In rodents, the depression-like aspects of nicotine withdrawal are reflected in elevated intracranial self-stimulation (ICSS) reward thresholds (Epping-Jordan et al., 1998). The

present study aimed to evaluate whether agonism or antagonism at GABA_B receptors would ameliorate the depression-like aspects of nicotine withdrawal by assessing the effects of a GABA_B receptor agonist, an antagonist, and a positive allosteric modulator on nicotine withdrawal-induced elevations in ICSS reward thresholds.

2. Materials and Methods

2.1. Subjects

Male Wistar rats (Charles River, Raleigh, NC) weighing 300–350 g upon arrival in the laboratory were group housed on a 12 h/12 h reverse light/dark cycle with unrestricted access to water except during testing. Behavioral testing occurred during the dark phase of the light/dark cycle. All subjects, animal facilities, and experimental protocols were in accordance with National Institutes of Health (National Research Council, 1996) and Association for the Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the Institutional Animal Research Committee.

2.2. Drugs

(–)Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in saline (pH adjusted to 7.0 ± 0.5 with sodium hydroxide). The solution was then filtered through a 0.22 μm syringe filter (Fisher Scientific, Pittsburgh, PA) for sterilization. Nicotine doses are reported as free base concentrations, while all doses for GABA_B receptor ligands are reported as salt concentrations. 3-amino-2[*S*]-hydroxypropyl)-methylphosphinic acid (CGP44532; compound #63 in (Froestl et al., 1995), [3-{1-(*S*)-[3-(cyclohexylmethyl)hydroxy]phosphinyl}-2-(*S*) hydroxypropyl]amino}ethyl]benzoic acid (CGP56433A; Table IV in (Froestl et al., 1996), and *N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine [(BHF177; compound #27 in (Guery et al., 2007)] were synthesized and provided by SG and WF or DB and MGF. In GTPy³⁵S assays on CHO-K1 membranes from GABA-B_(1b/2) co-expressing cells, the potency of BHF177 was 1.7 μM (measured at 1 μM GABA; Guery et al. 2007), similar to that of other positive modulators [CGP7930 and GS39783; Urwyler et al., 2001; Urwyler et al., 2003], although BHF177 is structurally different than CGP7930 and GS39783. CGP44532 and CGP56433A were dissolved in 0.9% saline and administered subcutaneously (1 ml/kg, 30 min pretreatment time). BHF177 was suspended in 0.5% methylcellulose and administered intraperitoneally (2 ml/kg, 30 min pretreatment time). The different routes of administration used in these experiments were selected for direct comparisons with previous studies assessing the effects of GABA_B receptor agonists and antagonists on performance in the ICSS task (Macey et al., 2001; Paterson et al., 2008b).

2.3. Apparatus

Sixteen Plexiglas chambers were used (30.5 × 30 × 17 cm; Med Associates, St. Albans, VT), each housed in a sound-attenuating box (San Diego Instruments, San Diego, CA). Each chamber contained a metal wheel manipulandum (5 cm wide), centered in a side wall, that required ~ 0.2 N force for a quarter turn rotation. Brain stimulation was delivered by constant current stimulators (Stimtech 1200, San Diego Instruments, San Diego, CA). Subjects were connected to the stimulation circuit with bipolar leads (Plastics One, Roanoke, VA) attached to gold-contact swivel commutators (model SL2C, Plastics One, Roanoke, VA).

2.4. ICSS electrode placement

Rats were anesthetized with an isoflurane/oxygen vapor mixture (1–1.5%) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Stainless steel bipolar electrodes

(11 mm, model MS303/2, Plastics One, Roanoke, VA) were implanted in the posterior lateral hypothalamus (anterior/posterior, -0.5 mm from bregma; medial/lateral, ± 1.7 mm; dorsal/ventral, -8.3 mm from dura; with the incisor bar 5 mm above the interaural line; (Paxinos and Watson, 1998).

2.5. Osmotic minipump implantation

Rats were anesthetized with an isoflurane/oxygen vapor mixture (1–1.5%), and an osmotic minipump (models 2ML1 and 2ML2, Alza Corp., Palo Alto, CA, USA) was inserted subcutaneously, as described previously (Der-Avakian and Markou, 2010; Paterson et al., 2007). Minipumps were removed on day 7 (Experiments 1 and 2) or day 14 (Experiment 3) under anesthesia.

2.6. Behavioral procedures

2.6.1. ICSS training—The discrete-trial current-threshold procedure was a modification of a task initially developed by Kornetsky and Esposito (1979) and described in detail elsewhere (Markou and Koob, 1992). The rats were first trained to turn the wheel manipulandum on a fixed-ratio 1 (FR1) schedule of reinforcement. Each quarter turn of the wheel resulted in the delivery of a 500 ms train of 0.1 ms cathodal square-wave pulses at a frequency of 100 Hz. After successful acquisition of responding for stimulation on this FR1 schedule (100 reinforcements within 10 min), the rats were trained gradually on the discrete-trial current-threshold procedure.

Each trial began with the delivery of a noncontingent electrical stimulus, followed by a 7.5 s response window within which the subject could make a response to receive a second contingent stimulus identical to the initial noncontingent stimulus. A response during this time window was labeled a positive response, whereas the lack of a response was labeled a negative response. During a 2 s period immediately after a positive response, additional responses had no consequence. The intertrial interval that followed either a positive response or the end of the response window in the case of a negative response had an average duration of 10 s (ranging from 7.5 s to 12.5 s). Responses that occurred during the intertrial interval were labeled timeout responses and resulted in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the intertrial interval and delay periods induced by timeout responses were gradually increased until animals performed consistently for a fixed stimulation intensity at standard test parameters. The animals were subsequently tested on the current-intensity threshold procedure in which stimulation intensities were varied according to the classical psychophysical method of limits. A test session consisted of four alternating series of descending and ascending current intensities starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the current intensity was changed by 5 μ A steps between blocks of trials. The initial stimulus intensity was set at approximately 40 μ A above the baseline current-intensity threshold for each animal. Each test session typically lasted 30–40 min and provided two dependent variables for behavioral assessment: threshold and response latency.

Threshold: The current-threshold for each descending series was defined as the stimulus intensity between the successful completion of a set of trials (positive responses during two or more of the three trials) and the stimulus intensity for the first set of trials, of two consecutive sets, during which the animal failed to respond positively on two or more of the three trials. The current-intensity threshold for each ascending series was defined as the stimulus intensity between a current intensity in which the animal failed to respond positively on two or more of the three trials and the first set of trials, of two consecutive sets,

during which the animal responded positively on two or more of the three trials. The mean of the four series' thresholds was defined as the threshold for the session.

Response latency: The time interval between the beginning of the noncontingent stimulus and a positive response was recorded as the response latency. The response latency for each session was defined as the mean response latency on all trials during which a positive response occurred.

2.6.2. Experimental procedures

Experiment 1. Effects of the GABA_B receptor agonist CGP44532 on nicotine withdrawal assessed by ICSS threshold elevations: After successful acquisition of stable ICSS thresholds (less than 10% variation over 3 baseline days), naive rats were prepared with minipumps containing either nicotine or saline (3.16 mg/kg/day nicotine base). Seven days later, pumps were removed under anesthesia, and ICSS thresholds were assessed 6 h later and at 24 h intervals thereafter. Rats were assigned to six groups ($n = 9-11$ /group), counter-balanced for ICSS threshold elevations (for the three nicotine-treated groups) at the 6 h post-pump time-point (three dose groups per pump content [nicotine or saline]). CGP44532 (0, 0.25, and 0.5 mg/kg, s.c.) was administered according to a between-subjects design 30 min prior to ICSS testing at the 30 h post-pump time-point. Thresholds continued to be assessed at 24 h intervals until thresholds returned to pre-nicotine/saline baseline levels.

Experiment 2. Effects of the GABA_B receptor antagonist CGP56433A on nicotine withdrawal assessed by ICSS threshold elevations: The experimental design of this study was identical to that of Experiment 1 described above. CGP56433A (0, 5, and 10 mg/kg, s.c.) was administered according to a between-subjects design. Thus, similar to Experiment 1, Experiment 2 had six groups, with 8-10 rats/group.

Experiment 3. Effects of the GABA_B receptor positive modulator BHF177 on nicotine withdrawal assessed by ICSS threshold elevations: The experimental design of this study was identical to that of Experiments 1 and 2 described above, except that BHF177 (0, 7.5, and 15 mg/kg, i.p.) was administered according to a between-subjects design, and rats were exposed to nicotine/saline for 14 instead of 7 days. Similar to Experiments 1 and 2, Experiment 3 had six groups, with 8-11 rats/group. The longer nicotine exposure was intended to induce a more prolonged or larger magnitude of threshold elevations than the one seen in Experiments 1 and 2 (Skjei and Markou, 2003).

2.7. Data analyses

Data are expressed as a percentage of the mean of the three baseline values before the implantation of the minipumps (i.e., to assess the effects during the nicotine administration phase). To most accurately represent the withdrawal effects and the effects of manipulations, we expressed the nicotine withdrawal phase data as a percentage of the baseline values obtained on the last day before pump removal. Data were analyzed using appropriate two-way mixed-factor analyses of variance (ANOVAs). Significant main or interaction effects were followed by one- or two-way ANOVAs on data from specific time-points post-pump removal and Newman-Keuls *post hoc* tests. Values of $P < 0.05$ were considered statistically significant. Statistical analyses were conducted using the Statistical Package for the Social Sciences v.16.0 and GraphPad Prism 6.0.

3. Results

3.1. Experiment 1: Effects of the GABA_B receptor agonist CGP44532 on nicotine withdrawal

3.1.1. Nicotine administration phase—In nicotine-exposed rats, mean (\pm SEM) baseline ICSS thresholds and latencies were $96.7 \pm 2.9 \mu\text{A}$ and $3.2 \pm 0.1 \text{ s}$, respectively. In saline-exposed rats, mean (\pm SEM) thresholds and latencies were $97.6 \pm 4.4 \mu\text{A}$ and $3.3 \pm 0.1 \text{ s}$, respectively. Administration of nicotine or saline via osmotic minipumps did not affect ICSS thresholds, whereas response latencies were shorter during the 7 days of exposure (*Nicotine*: $F_{1,49} = 4.89$, $P < 0.05$). No significant interaction effects were observed on thresholds or response latencies.

3.1.2. Nicotine withdrawal phase—A two-way repeated measures ANOVA on ICSS thresholds obtained during the 5 days after minipump removal (*Pump*: two levels; *CGP44532 Dose*: three levels) indicated that nicotine withdrawal was associated with significantly elevated ICSS thresholds (*Pump*: $F_{1,57} = 22.92$, $P < 0.0001$) that returned to baseline values over the 5 days of testing (*Time*: $F_{4,172} = 25.83$, $P < 0.0001$; *Pump* \times *Time*: $F_{4,172} = 9.91$, $P < 0.0001$). A follow-up one-way ANOVA on data at the 6 h post-pump removal time-point indicated that all nicotine-treated groups exhibited significantly elevated thresholds compared with rats exposed to saline-containing minipumps (main effect of *Pump*: $F_{1,53} = 39.09$, $P < 0.001$). A follow-up two-way ANOVA on data from the 30 h post-pump removal time-point showed that administration of CGP44532 elevated ICSS thresholds in all groups (*CGP44532 Dose*: $F_{2,54} = 7.73$, $P < 0.05$), and a significant difference was also observed between the saline- and nicotine-exposed rats at the same time-point (main effect of *Pump*: $F_{1,53} = 31.38$, $P < 0.0001$). No *Pump* \times *CGP44532 Dose* interaction was observed, indicating that the effects of CGP44532 were independent of whether rats were previously exposed to nicotine or saline. *Post hoc* comparisons indicated that nicotine-treated rats receiving 0.25 and 0.5 mg/kg CGP44532 exhibited significantly elevated ICSS thresholds compared with the saline/0 mg/kg CGP44532-treated control group ($P < 0.001$ and $P < 0.01$, respectively), saline/0.25 mg CGP44532 group ($P < 0.01$), and saline/0.5 mg CGP44532 group at the 30 h time-point ($P < 0.01$ and $P < 0.05$, respectively), respectively; Fig. 1). Nicotine-exposed rats receiving 0.25 mg/kg CGP44532 showed elevated ICSS thresholds compared with saline-exposed rats receiving 0.25 mg/kg CGP44532 at 54 h and 78 h after pump removal ($P < 0.001$, $P < 0.05$, respectively). Analysis of the response latency data indicated no effects of any of the manipulations on response latencies (data not shown).

3.2. Experiment 2: Effects of the GABA_B receptor antagonist CGP56433A on nicotine withdrawal

3.2.1. Nicotine administration phase—In nicotine-exposed rats, mean (\pm SEM) baseline thresholds and latencies were $106.5 \pm 5.3 \mu\text{A}$ and $3.2 \pm 0.1 \text{ s}$, respectively. In saline-exposed rats, mean (\pm SEM) baseline thresholds and latencies were $102.5 \pm 6.7 \mu\text{A}$ and $3.0 \pm 0.1 \text{ s}$, respectively. Administration of nicotine or saline via minipumps did not affect ICSS thresholds or response latencies. Only a gradual small decrease in thresholds was observed in both the saline and nicotine groups over the 7 days of saline or nicotine exposure (main effect of *Time*: $F_{6,53} = 2.22$, $P < 0.05$).

3.2.2. Nicotine withdrawal phase—A two-way repeated-measures ANOVA (*Pump*: two levels; *CGP56433A Dose*: three levels) on the threshold data obtained during the 5 days after minipump removal indicated that nicotine withdrawal was associated with significantly elevated ICSS thresholds (*Pump*: $F_{1,52} = 6.50$, $P < 0.05$) that returned to baseline levels over the 5 days of testing (*Time*: $F_{4,208} = 20.91$, $P < 0.0001$; *Pump* \times *Time*: $F_{4,208} = 5.25$, $P <$

0.001). A follow-up one-way ANOVA on data from the 6 h post-pump removal time-point indicated that nicotine-treated rats exhibited significantly elevated thresholds compared with saline-treated rats (main effect of *Pump*: $F_{1,48} = 28.11$, $P < 0.0001$). A follow-up two-way ANOVA on data from the 30 h post-pump removal time-point showed that administration of CGP56433A elevated ICSS thresholds in all groups (*CGP56433A Dose*: $F_{2,48} = 8.92$, $P < 0.001$), and a significant difference was also observed between the saline- and nicotine-treated rats (main effect of *Pump*: $F_{1,48} = 7.26$, $P < 0.01$). No significant *Pump* × *CGP56433A Dose* interaction effect was observed, indicating that the effects of CGP56433A were independent of whether rats were previously exposed to nicotine or saline. *Post hoc* comparisons revealed that ICSS thresholds in nicotine-exposed subjects receiving 10 mg/kg CGP56433A were significantly elevated compared with thresholds in saline- and nicotine/0 mg/kg CGP56433A rats ($P < 0.001$), saline/5 mg/kg CGP56433A rats ($P < 0.01$), saline/10 mg CGP56433A rats ($P < 0.01$), and nicotine/5 mg CGP56433A rats ($P < 0.05$) at the 30 h time-point (Fig. 2). No effects of any of the manipulations were observed on response latencies (data not shown).

3.3. Experiment 3: Effects of the GABA_B receptor positive modulator BHF177 on nicotine withdrawal

3.3.1. Nicotine administration phase—In nicotine-exposed rats, mean (\pm SEM) baseline thresholds and latencies were $97.4 \pm 4.9 \mu\text{A}$ and 3.0 ± 0.1 s, respectively. In saline-exposed rats, mean (\pm SEM) baseline thresholds and latencies were $93.5 \pm 7.6 \mu\text{A}$ and 3.1 ± 0.2 s, respectively. Administration of nicotine or saline via minipumps for 14 days did not affect ICSS thresholds. Response latencies were shorter (main effect of *Time*: $F_{13,49} = 4.068$, $P < 0.001$) during the 14 days of pump exposure, regardless of pump content.

3.3.2. Nicotine withdrawal phase—A two-way ANOVA on ICSS thresholds obtained during the 5 days after minipump removal (*Pump*: two levels; *BHF177 Dose*: three levels) indicated that nicotine withdrawal was associated with significantly elevated ICSS thresholds (*Pump*: $F_{1,49} = 10.64$, $P < 0.01$) that returned to baseline levels over the 5 days of testing (*Time*: $F_{4,196} = 12.55$, $P < 0.0001$; *Pump* × *Time*: $F_{4,196} = 6.81$, $P < 0.0001$). A follow-up one-way ANOVA on the 6 h post-pump removal time-point data showed that nicotine withdrawal significantly elevated ICSS thresholds compared with saline “withdrawal” ($F_{1,45} = 20.00$, $P < 0.0001$). A follow-up two-way ANOVA on data from the 30 h post-pump removal time-point showed that nicotine withdrawal continued to elevate thresholds at this time-point (main effect of *Pump*: $F_{1,45} = 8.84$, $P < 0.01$). Administration of BHF177 at the 30 h time-point exacerbated the already elevated ICSS thresholds induced by nicotine withdrawal (*BHF177 Dose*: $F_{2,45} = 4.03$, $P < 0.05$). *Post hoc* comparisons indicated that ICSS thresholds in nicotine-exposed subjects receiving 15 mg BHF177 were significantly greater than in the saline/0 mg/kg BHF177-treated group at the 30 h time-point post-pump removal ($P < 0.05$; Fig. 3). Analysis of response latency data indicated that response latencies were not affected by any of the manipulations (data not shown).

4. Discussion

The present studies showed that administration of a GABA_B receptor agonist, antagonist, or positive allosteric modulator exacerbated the elevated ICSS thresholds observed during nicotine withdrawal in rats. These threshold elevations during nicotine withdrawal are hypothesized to reflect an anhedonic state, and are consistent with results from previous studies in rodents (Epping-Jordan et al., 1998; Harrison et al., 2001; Johnson et al., 2008; Paterson et al., 2008a; Rylkova et al., 2008; Stoker et al., 2008). Both doses of the GABA_B receptor agonist CGP44532 (0.25 and 0.5 mg/kg), and the highest doses of either the GABA_B receptor antagonist CGP56433A (10 mg/kg) or the GABA_B receptor positive

modulator BHF177 (15 mg/kg) exacerbated the elevated ICSS thresholds seen during nicotine withdrawal. The effects of CGP56433A and BHF177 in nicotine-treated rats were short-lived, but ICSS thresholds remained elevated in nicotine-, but not saline-, exposed rats that received 0.25 mg/kg CGP44532, 24–48 h after CGP44532 administration (54–78 h post-nicotine). Thus, all GABA_B receptor ligands elevated ICSS reward thresholds in both nicotine- and saline-exposed rats, as reflected by the lack of a statistically significant interaction between nicotine/saline exposure and GABA compound dose.

It is possible that the results are due to a variety of non-specific effects of the compounds. However, administration of doses of CGP44532, CGP56433A or BHF177 that significantly exacerbated the elevated ICSS thresholds seen during nicotine withdrawal did not affect the animals' performance, as reflected in lack of effects on response latencies. This lack of non-specific effects is consistent with previous findings showing lack of effects of GABA_B compounds on response latencies in the ICSS procedure under baseline conditions or during nicotine-induced facilitation of brain reward function (Macey et al., 2001; Paterson et al., 2008b). These findings indicate that there were no sedative or muscle relaxant effects of the compounds that could have interfered with task performance.

One could also speculate that the nicotine withdrawal/ICSS model of anhedonia is not suitable for detecting the effects of GABA compounds. However, this possibility is rather unlikely, because this model has been shown to reliably detect antidepressant-like properties of a variety of compounds, such as selective serotonin reuptake inhibitors (Harrison et al. 2001), the tricyclic antidepressant desipramine (Paterson et al 2008a), the atypical antidepressant bupropion (Cryan et al., 2003a; Paterson 2007), as well as the effects of novel compounds that have not been shown yet to have antidepressant properties in humans, such as a Group II metabotropic glutamate receptor antagonist (Kenny et al., 2003), and an alpha₂ adrenergic receptor antagonist (Semenova and Markou, 2010). Thus, nicotine-induced elevations in brain reward thresholds is an animal model of depression-like behavior that is sensitive to the effects of a variety of antidepressant or putative antidepressant treatments that act through various neurotransmitter systems and targets.

The similar behavioral effects of the GABA_B receptor agonists, antagonists and positive modulators on the depressive-like aspect of nicotine withdrawal are unexpected. Nevertheless, many other studies have also reported similar effects of GABA_B agonists and antagonists. Specifically, both the GABA_B receptor agonist CGP44532 and the GABA_B receptor antagonist CGP56433A elevated ICSS thresholds in rats under baseline conditions (Macey et al., 2001). Furthermore, the GABA_B receptor positive modulator CGP7930 and the GABA_B receptor antagonist SCH50911 showed antidepressant-like profiles in the forced swim test in mice, similar to the GABA_B receptor agonists baclofen and SKF 97541 (also termed CGP35024; (Chebib et al., 1997), which also exhibits GABA_C receptor antagonist properties (Frankowska et al., 2007). GABA_B receptor positive modulators and antagonists also exhibited similar effects in the elevated zero maze, while neither of them was effective in the modified Geller-Seifter task, both tests of anxiety-like behavior (Frankowska et al., 2007; but see Paterson and Hanania, 2010).

Furthermore, both the GABA_B receptor agonist baclofen and the GABA_B receptor antagonist CGP35348 reduced the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized animals (Sahraei et al., 2009). Additionally, GABA_B receptor agonists and antagonists exhibited similar behavioral effects in tests of cognitive function, such as passive avoidance, a fear-motivated avoidance task that assesses memory (Castellano et al., 1993; Escher and Mittleman, 2004; Mondadori et al., 1993). Despite impaired performance in a spatial working memory task exhibited by GABA_B receptor knockout mice (Jacobson et al., 2007b), GABA_B receptor antagonists

improved working memory, reflected by improved pattern recognition speed and attention, reflected by choice reaction time and visual information processing, in humans (Froestl et al., 2004), as well as performance in learning and memory tasks (Getova and Bowery, 1998; Helm et al., 2005; Nakagawa and Takashima, 1997) in rodents and non-human primates.

Altogether, these findings indicate that opposing actions of GABA_B receptor agonists and antagonists had effects in the same direction in various behavioral studies, including antidepressant-like and anxiolytic-like properties, exacerbation of the depressive-like state of nicotine withdrawal, decreased brain stimulation and morphine reward, and improved performance in cognitive tasks.

Based on the above findings, it is likely that GABA_B receptor agonists, antagonists and modulators induce the same direction of effects by differentially binding to the various GABA_B receptors. Specifically, GABA_B receptors are high-molecular-mass complexes, consisting of two main subunits, GABA_{B1} and GABA_{B2}, and auxiliary subunits, such as potassium channel tetramerization domain-containing (KCTD) proteins (Bartoi et al., 2010). KCTD proteins associate tightly with the carboxy terminus of the GABA_{B2} subunit as tetramers, thus increasing agonist potency and altering G-protein signaling by accelerating signaling onset and promoting receptor desensitization (Schwenk et al., 2010). The GABA_B receptor isoforms GABA_{B1a} and GABA_{B1b} (Kaupmann et al., 1997) combine with the GABA_{B2} subunit to form a functional receptor (Jones et al., 1998; Kaupmann et al., 1998; Kuner et al., 1999; Mohler and Fritschy, 1999; Ng et al., 1999; White et al., 1998). The GABA_{B1a/2} isoform is mainly found presynaptically as a heteroreceptor that regulates glutamate, dopamine, and serotonin transmission (Vigot et al., 2006), whereas the GABA_{B1b/2} isoform is located primarily postsynaptically. Both GABA_{B1a/2} and GABA_{B1b/2} isoforms are also presynaptic autoreceptors that inhibit neurotransmitter release through inhibition of Ca²⁺ channels. Importantly, both pre- and postsynaptic GABA_B receptors are expressed in brain reward circuitries and are hypothesized to be involved in reward processes (e.g. Bischoff et al., 1999; Brebner et al., 1999; Corrigan et al., 2000; Fattore et al., 2002; Frankowska et al., 2008; Paterson et al., 2004; Xi et al., 2003).

Based on the localization of the different GABA_B receptor subtypes (Bettler and Tiao, 2006; Vigot et al., 2006), it is possible that the results are due to differential binding of the GABA_B receptor ligands on these receptor subtypes. For example, the GABA_B receptor agonist CGP44532 may have further increased the hypothesized increased GABAergic neurotransmitter function associated with nicotine withdrawal by possibly activating postsynaptic GABA_{B1b/2} receptors. The GABA_B receptor positive modulator BHF177 may have acted similarly, by binding to the GABA_{B2} subunit of the GABA_B receptor (Binet et al., 2004; Cryan and Kaupmann, 2005; Dupuis et al., 2006) at postsynaptic sites. By contrast, the GABA_B receptor antagonist CGP56433A is hypothesized to have acted by blocking primarily presynaptic autoreceptors of either the GABA_{B1a/2} or GABA_{B1b/2} isoform on GABAergic terminals, thus further increasing GABA release during nicotine withdrawal. Importantly, based on the recently demonstrated heterogeneity of GABA_B receptors and their multi-subunit structure, the effects of the tested GABA_B receptor compounds on GABA_B receptors could also be affected by the effects of KCTD auxiliary subunits on the GABA_{B2} receptor subunit (Bartoi et al., 2010; Schwenk et al., 2010). Several behavioral effects summarized above, showing that opposing actions of GABA_B receptor agonists, antagonists and modulators had effects in the same direction, support this speculative hypothesis of differential binding of the GABA_B receptor ligands on the different GABA_B receptor subtypes (e.g. Frankowska et al., 2007; Macey et al., 2001).

5. Conclusions

In summary, both GABA_B receptor activation and blockade aggravated the anhedonic state of nicotine withdrawal in rats. Thus, these compounds did not exhibit antidepressant-like effects in the nicotine withdrawal/ICSS model of anhedonia (Barr and Markou, 2005) and, therefore, may not be useful therapeutics for the treatment of the depression-like state and anhedonia associated with nicotine withdrawal.

None of the compounds used in the present studies are known to be selective for specific GABA_B receptor isoforms or for pre- vs. postsynaptic GABA_B receptors. The unidirectional effects of GABA_B receptor activation and blockade may reflect differential efficacy of these compounds at presynaptic hetero- and autoreceptors compared with postsynaptic GABA_B receptors. The development of more selective GABA_B receptor subtype ligands will allow the delineation of the role of these GABA_B receptor isoforms in reward-related processes and in the anhedonic aspects of nicotine withdrawal and depression.

Acknowledgments

This work was supported by National Institutes of Health grant U19DA026838 to AM. SV was supported by an Individual Postdoctoral Research Fellowship from the Tobacco-Related Disease Research Program from the State of California (18FT-0048). The authors wish to thank Mr. Michael Arends for editorial support, and Mrs. Kim Edwards and Mrs. Jessica Benedict for technical assistance.

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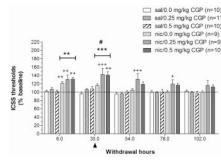


Figure 1. Effects of the GABA_B receptor agonist CGP44532 on ICSS thresholds during nicotine/saline withdrawal

Data are expressed as the percentage of baseline thresholds obtained on the last day of pump exposure before removal of the minipump (mean \pm SEM). The arrowhead indicates the time-point (30 h post-pump removal) at which the GABA_B receptor agonist CGP44532 was administered. Asterisks (** $P < 0.01$, *** $P < 0.001$) indicate significantly elevated ICSS thresholds in nicotine-treated groups compared with the saline/0 mg/kg CGP44532-treated group. Plus signs (** $P < 0.01$, *** $P < 0.001$) indicate significant differences between each nicotine-treated group compared with its corresponding saline-treated group that received the same dose of CGP44532 (Newman-Keuls *post hoc* test). The pound sign ($\#P < 0.05$) indicates a significant main effect of CGP44532 at the 30 h time-point.

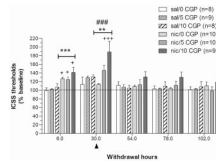


Figure 2. Effects of the GABA_B receptor antagonist CGP56433A on ICSS performance during nicotine/saline withdrawal

Data are expressed as the percentage of baseline thresholds obtained on the last day of pump exposure before removal of the minipump (mean \pm SEM). The arrowhead indicates the time-point (30 h post-pump removal) when the GABA_B receptor antagonist CGP56433A was administered. Asterisks (** $P < 0.01$, *** $P < 0.001$) indicate statistically significant elevations in ICSS thresholds in nicotine-treated groups compared with the saline/0 mg/kg CGP56433A-treated group. Plus signs (+ $P < 0.05$, ++ $P < 0.001$) indicate significant differences between each nicotine-treated group compared with its corresponding saline-treated group that received the same dose of CGP56433A and compared with the nicotine/vehicle-treated group at the 30 h time-point (Newman-Keuls *post hoc* test). The pound signs (### $P < 0.01$) indicate a significant main effect of CGP44532 at the 30 h time-point.

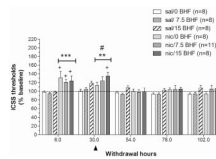


Figure 3. Effects of the GABA_B receptor positive modulator BHF177 on ICSS performance during nicotine/saline withdrawal

Data are expressed as the percentage of baseline thresholds obtained on the last day of pump exposure before removal of the minipump (mean \pm SEM). The arrowhead indicates the time-point (30 h post-pump removal) when the GABA_B receptor positive modulator BHF177 was administered. Asterisks (** $P < 0.01$, *** $P < 0.001$) indicate significantly elevated ICSS thresholds in nicotine-treated groups compared with the saline/0 mg/kg BHF177-treated group. Plus signs (+ $P < 0.05$) indicate significant differences between each nicotine-treated group and its corresponding saline-treated group that received the same dose of BHF177 (Newman-Keuls *post hoc* test). The pound sign (# $P < 0.05$) indicates a significant main effect of BHF177.