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Metabotropic glutamate 2/3 receptor activation induced reward deficits but did not aggravate brain reward deficits associated with spontaneous nicotine withdrawal in rats

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ABSTRACT

Glutamate neurotransmission, and particularly metabotropic glutamate (mGlu) 2/3 receptors are implicated in behaviors of relevance to the addictive properties of nicotine. In laboratory animals, the mGlu2/3 receptor agonist LY379268 has been previously shown to decrease intravenous nicotine self-administration and cue-induced reinstatement of nicotine-seeking behavior. Such mGlu2/3 receptor agonists may therefore be useful medications to assist people in smoking cessation. Because of the demonstrated preclinical efficacy of mGlu2/3 receptor agonists in decreasing the primary rewarding and conditioned effects of nicotine in rats, we wished to examine whether such compounds could potentially influence additional aspects of nicotine dependence, such as nicotine withdrawal. We hypothesized that an mGlu2/3 receptor agonist would have negative effects on nicotine withdrawal because mGlu2/3 receptor antagonists have previously been shown to attenuate nicotine withdrawal-induced reward deficits, while an mGlu2/3 receptor agonist precipitated withdrawal-like reward deficits in rats dependent on nicotine. To test this hypothesis, we assessed the effects of the mGlu2/3 receptor agonist LY379268 on brain reward deficits associated with spontaneous nicotine withdrawal in rats. Brain reward function, as assessed by intracranial self-stimulation reward thresholds, was examined after removal of nicotine- or saline-delivering subcutaneous osmotic minipumps. LY379268 administration produced reward deficits in animals "withdrawing" from chronic saline administration and only tended to aggravate nicotine withdrawal-induced reward deficits in rats previously treated with nicotine. Thus, this mGlu2/3 agonist does not appear to significantly influence the affective depression-like aspects of nicotine withdrawal.

1. Introduction

Nicotine withdrawal produces negative affective symptoms in humans, including depressed mood, irritability, craving, and anxiety [1–3]. This aversive abstinence syndrome in smokers is thought to contribute to the persistence of the tobacco smoking habit and relapse during abstinence [4,5]. In animals, nicotine withdrawal precipitates a deficit in brain reward function, as measured by elevations in reward thresholds for intracranial self-stimulation (ICSS), similar to those observed in rats undergoing withdrawal from other major drugs of abuse [6]. In addition, a somatic nicotine withdrawal syndrome has been characterized in rats, including somatic signs such as abdominal constrictions (writhes), gasps, foot licks, and eyeblinks [6–8].

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Metabotropic glutamate (mGlu) 2/3 receptors are presynaptic inhibitory autoreceptors that negatively modulate excitatory glutamate transmission [9]. LY379268 is a heterobicyclic amino acid and selective mGlu2/3 receptor agonist. LY379268 is highly selective for the presynaptic mGlu2 and mGlu3 receptor subtypes as compared to other glutamate receptors including *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainate, and other mGlu receptors [10].

mGlu2/3 receptor agonists reduce brain glutamate transmission, thereby indirectly reducing dopamine levels in the nucleus accumbens [11]. Both reduced glutamate and dopamine transmission play a role in depression and withdrawal from drugs of abuse [12–14]. Accordingly, mGlu2/3 receptor antagonists that increase glutamate and dopamine neurotransmission have been suggested as potential treatments for depression and depressive symptoms associated with drug-withdrawal [13,14]. Consistent with this hypothesis, mGlu2/3 receptor antagonists had antidepressant-like effects in the rat forced swim test [15], the mouse tail suspension test [15], and the learned helplessness procedure in rats [16], prevented stress-induced autonomic hyperactivity [17], and attenuated reward deficits associated with spontaneous nicotine withdrawal in rats [18]. In contrast, the mGlu2/3 receptor agonist LY314582 precipitated withdrawal-like reward deficits in nicotine-dependent rats while nicotine was on board [18]. Taken together, these data suggest that mGlu2/3 receptor antagonists may be used to treat depression and depressive symptoms during nicotine withdrawal.

However, mGlu2/3 receptor agonists have also been suggested for the treatment of drug dependence based on their well-documented potential to reduce drug reward and reinstatement of drug-seeking behavior that was previously extinguished. In particular, the mGlu2/3 receptor agonist LY379268 reduced self-administration of cocaine, ethanol, and nicotine and prevented reinstatement of drug-seeking behavior for cocaine, ethanol, heroin, and nicotine [19–25]. While many recent studies have assessed the beneficial effects of the mGlu2/3 receptor agonist LY379268 in decreasing drug reward and drug-seeking behavior, little if any work has assessed whether mGlu2/3 receptor stimulation may have adverse effects on the reward system during spontaneous drug withdrawal. Because the mGlu2/3 receptor antagonist LY341495 reversed brain reward deficits associated with nicotine withdrawal and the mGlu2/3 agonist LY314582 precipitated nicotine withdrawal-like reward deficits in rats under chronic nicotine treatment, we hypothesized that the mGlu2/3 receptor agonist LY379268 would increase reward deficits associated with spontaneous nicotine withdrawal in rats.

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 (two per cage throughout the duration of the study) in a temperature- and humidity-controlled vivarium on a 12 h reverse light–dark cycle (lights off at 8 a.m.). All behavioral testing took place during the dark phase of the light–dark cycle. After arrival in the vivarium, animals were allowed to habituate to their new environment for 1 week and were handled twice during that time. Rats had unrestricted access to food and water except during testing. Animal care and experimental protocols were in accordance with the NIH guidelines and the Association for the Assessment of Accreditation of Laboratory Animal Care (AAALAC), and approved by the institutional committee.

2.2. Drugs

(−)Nicotine hydrogen tartrate was purchased from Sigma (St. Louis, MO), dissolved in saline, and administered continuously using subcutaneous osmotic minipumps delivering 9 mg/kg/day nicotine hydrogen tartrate (3.16 mg/kg/day nicotine base) for 10 days. LY379268 [(−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate] was custom synthesized according to the synthesis described in [10] and purchased from ANAWA (Wangen, Switzerland). LY379268 was dissolved in sterile water, pH adjusted with NaOH, and administered subcutaneously at 1 or 3 mg/kg (salt) in a volume of 1 mg/ml or 3 mg/ml, respectively, 30 min before ICSS testing sessions.

2.3. Intracranial self-stimulation testing chambers

Intracranial self-stimulation training and testing took place in 16 sound-attenuated Plexiglas experimental chambers (30.5 cm × 30 cm × 17 cm; Med Associates, St. Albans, VT). One wall contained a metal wheel manipulandum (5 cm wide), which required 0.2 N force to rotate it a quarter turn. Brain stimulation was delivered by constant current stimulators (Stimtech 1200; San Diego Instruments, San Diego, CA). Subjects were connected to the stimulation circuit through flexible bipolar leads (Plastics One) attached to gold-contact swivel commutators (model SL2C; Plastics One, Roanoke, VA). The stimulation parameters, data collection, and all test session functions were controlled by a microcomputer.

2.4. Intracranial self-stimulation electrode implantation

Rats were anesthetized with an isoflurane/oxygen mixture (1–3% isoflurane) and positioned in a stereotaxic frame (David Kopf Instruments, Tujanga, CA) with the incisor bar set 5 mm above the interaural line. Stainless steel bipolar electrodes (model MS303/2, Plastics One), 11 mm in length, were implanted into the medial forebrain bundle at the level of the posterior lateral hypothalamus (AP −0.5 mm from bregma; ML ± 1.7 mm; DV −8.3 mm from dura [26]). Animals were allowed at least 7 days to recover from surgery prior to any behavioral training.

2.5. Osmotic minipump implantation and removal

Rats were anesthetized with an isoflurane/oxygen mixture (1–3% isoflurane) and an osmotic minipump (Alzet model 2ML2, 10 μl/h, 14 days; Durect Corporation, Cupertino, CA) was inserted subcutaneously (back of the animal, parallel to the spine) with the flow moderator directed posteriorly. Pumps were surgically removed 10 days later under isoflurane anesthesia.

2.6. Intracranial self-stimulation reward threshold procedure

The discrete-trial current-threshold procedure used was a modification of a task initially developed by Kornetsky and Esposito [27] and described in [28]. The rats were initially 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 the successful acquisition of responding for stimulation on this FR1 schedule, defined as 100 reinforcements within 10 min, the rats were trained gradually on the discrete-trial current-threshold procedure [28,29]. Each trial began with the delivery of a non-contingent 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 in all parameters to the initial non-contingent stimulus. A response during this time window was labeled a positive response, while the lack of a response was labeled a negative response. During a 2 s period immediately after a positive response, additional responses had no consequences (extra responses). The inter-trial interval (ITI), which 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 ITI were labeled time-out 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 ITI and delay periods induced by time-out 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-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 intensity changed by steps of 5 μA between blocks of trials. The initial stimulus intensity was set at approximately 40 μA above the baseline current-threshold for each animal. Each test session typically lasted 30 min and provided two dependent variables for behavioral assessment: threshold and response latency.

2.6.1. Threshold

The current-threshold for each series was defined as the midpoint in microamperes between the current intensity level at which the animal made two or more positive responses out of the three stimulus presentations and the level where the animal made less than two positive responses, at two successive stimulus intensities that led to this outcome. The animal's estimated current threshold for each test session was the mean of the four series' thresholds.

2.6.2. Response latency

The time interval between the initiation of the non-contingent stimulus and a positive response was recorded as the response latency. The response latency for each session was defined as the mean response latency of all trials during which a positive response occurred.

2.7. Ratings of somatic signs of nicotine withdrawal

Rats were placed individually in transparent plastic cylindrical containers (30 cm in diameter and height = 38 cm) in which they could move freely. Subjects were habituated to these containers for 5 min each day during three consecutive days before the first test session. During the test session, the rats were observed by one of three experienced observers that were blind to the experimental treatments. Each observer rated animals from all four treatment groups to decrease potential bias by inter-observer variability. Each rat was rated by only one observer. Rats were observed for 10 min, and the frequency of the following signs was recorded based on a checklist of nicotine abstinence signs [30]: body shakes, chews, cheek tremors, eye blinks, foot licks, gasps, genital licks, head shakes, ptosis, scratches, teeth chattering, writhes, and yawns. Multiple successive counts of any sign required a distinct pause between episodes. Ptosis, if present, was recorded only once per minute. For statistical analyses, the total number of somatic signs was defined as the sum of individual occurrences of the aforementioned withdrawal signs.

2.8. Experimental design

For all experiments, animals were surgically prepared with ICSS electrodes and trained in the intracranial self-stimulation procedure until stable baseline responding was achieved, defined as $<10\%$ variation in thresholds for three consecutive days. To achieve stable baseline ICSS thresholds, approximately 3 weeks of daily testing were required after the subjects had learned the procedure.

2.9. Effects of LY379268 on brain reward thresholds under baseline conditions

We first tested the effects of the mGlu2/3 receptor agonist LY379268 alone on intracranial self-stimulation brain reward threshold to evaluate the maximal dose of LY379268 that would not affect thresholds when given under baseline conditions. Rats ($n = 12$) were prepared with intracranial self-stimulation electrodes and trained in the self-stimulation procedure until stable thresholds were obtained. A within-subjects Latin square design was used to test the effect of two doses of LY379268 (1 and 3 mg/kg) or vehicle on reward thresholds. Animals were tested in the self-stimulation procedure twice each day (Session 1 and 2) with 30 min between sessions. On the three drug injection days, LY379268 or vehicle was injected after Session 1, 30 min before Session 2, to assess the effect of the compound/vehicle on reward thresholds during Session 2 compared to baseline values obtained during Session 1. Test days were separated by 48 h. On days between test days, animals were similarly tested twice a day, and vehicle was injected before Session 2.

2.10. Effect of LY379268 on nicotine withdrawal-induced brain reward threshold elevations and somatic signs of withdrawal

Naïve rats ($n = 29$) were prepared with electrodes and trained in the ICSS procedure until stable thresholds were obtained.

Animals were then surgically prepared with osmotic minipumps delivering either 9 mg/kg/day nicotine hydrogen tartrate ($n = 16$) or saline ($n = 13$). Pumps were removed after 10 days, and reward thresholds were measured 6, 12, 24, 30, 48, 72, 96, 120, and 144 h after pump removal. After the 6 h time-point assessments, rats were assigned to four treatment groups that were counterbalanced in terms of absolute mean thresholds during the last 3 days of exposure to the minipumps, and for the previously nicotine-treated rats, the percent elevation in thresholds observed at the 6 h time-point after removal of the nicotine-containing minipump. Thus, there were minimal differences in ''withdrawal'' thresholds between subgroups assigned to receive LY379268 and vehicle within the saline- and nicotine-treated groups. A 2×2 factorial design was used to assess the effects of LY379268/vehicle on reward thresholds during nicotine/saline withdrawal. Eight animals from the previously nicotine-treated group and seven animals from the previously saline-treated group were injected with 1 mg/kg LY379268 before the 12 h time-point and again with 3 mg/kg LY379268 before the 30 h time-point. The second higher 3 mg/kg LY379268 dose was used because, contrary to our prediction, we observed no effects of the first 1 mg/kg LY379268 dose in nicotine-withdrawing rats (see Section 3). Because nicotine withdrawal persists for at least 3 days after removal of the nicotine-containing minipumps, it was possible to assess the effects of this higher second dose of LY379268. Group assignments (vehicle versus drug) remained the same for all subjects, as potential carry-over effects of the first injection would not allow the use of a cross-over design, while still allowing the assessment of the overall effects of the mGlu2/3 receptor agonist on nicotine withdrawal. The remaining rats in each group ($n = 8$ nicotine-treated rats and $n = 6$ saline-treated rats) were injected with vehicle at the same time-points. Somatic signs of nicotine withdrawal were assessed immediately after the 12 h and the 30 h reward threshold assessments, which was approximately 90 min after administration of 1 or 3 mg/kg LY379268 and 13.5 or 31.5 h, respectively, after nicotine or saline pump removal.

2.11. Statistical analyses

All threshold data were expressed as percent of baseline thresholds. Thresholds after administration of LY379268 under baseline conditions during Session 2 were expressed as percent of the threshold of the preceding Session 1. Thresholds during nicotine/saline withdrawal were expressed as percent of the mean thresholds during the last 3 days of pump treatment. Effects of LY379268 on reward thresholds under baseline condition were analyzed using repeated-measures ANOVA with LY379268 Dose (0, 1, and 3 mg/kg) as the within-subjects factor. To assess the effect of LY379268 on nicotine withdrawal-induced reward threshold elevations, we conducted an overall repeated-measures ANOVA with Pump (nicotine versus saline) and Drug (LY379268 versus vehicle) as between-subjects factors and Time as a within-subject factor. To further test the effect of the different doses of LY379268, separate follow-up ANOVAs were performed on the 12 h (1 mg/kg) and 30 h (3 mg/kg) time-point threshold measurements because these were the time-points when animals were administered LY379268 or vehicle. Newman-Keuls tests were used for post hoc analysis. Finally, a two-way ANOVA was conducted on the withdrawal threshold data by calculating ''area under the curve'' by adding the percent thresholds of time points 6–144 h after pump removal; the two between-subjects factors were Pump (nicotine versus saline) and Drug (LY379268 versus vehicle). The area under the curve analysis was included to provide a summary view of the drug effects collapsed over time. Response latencies were analyzed using similar ANOVAs as for the threshold data. The effect of LY379268 (1 mg/kg) on somatic signs of nicotine withdrawal was similarly assessed using a two-way ANOVA with Pump and Drug as factors.

3. Results

3.1. Effect of LY379268 on brain reward thresholds under baseline conditions

Mean ± S.E.M. baseline thresholds during Session 1 were 106 ± 7 μA. LY379268 elevated intracranial self-stimulation thresholds [$F(2,22) = 4.8$, $p < 0.02$]. The dose of 3 mg/kg LY379268 significantly elevated reward thresholds ($p < 0.001$), while the 1 mg/kg dose induced a non-significant elevation (Fig. 1). Mean ± S.E.M. baseline response latencies during Session 1 were 3.36 ± 0.13 s before vehicle injection, 3.14 ± 0.12 s before the 1 mg/kg LY379268 dose injection, and 3.14 ± 0.15 s before the 3 mg/kg LY379268 dose administration. During Session 2, mean ± S.E.M. percent of Session 1 response latencies were 100.2 ± 2.5 after vehicle administration, 103.2 ± 2.2 after the administration of 1 mg/kg LY379268 and 103.6 ± 2.98 after administration of 3 mg/kg LY379268. LY379268 did not significantly affect mean response latencies.

3.2. Effect of LY379268 on nicotine withdrawal-induced brain reward threshold elevations and somatic signs of nicotine withdrawal

Mean ± S.E.M. baseline thresholds before the pump removal were 136.5 ± 16.8 μA for the nicotine/vehicle group, 133.6 ± 21.6 μA for the nicotine/LY379268 group, 154.6 ± 27 μA for the saline/vehicle group, and 123 ± 11.3 μA for the saline/LY379268 group. After removal of the minipumps, as expected, brain reward thresholds were significantly elevated in animals previously treated with nicotine compared to saline-treated controls [main effect of Pump: $F(1,23) = 53.9$; $p < 0.001$] indicating nicotine withdrawal-induced reward deficits. Treatment with LY379268 also significantly elevated thresholds [main effect of Drug $F(1,23) = 5.2$; $p < 0.05$] (Fig. 2A). Factorial ANOVA on the ''area under the curve'' data curves confirmed the results from the above analyses and showed a significant main effect of Pump [$F(1,24) = 36.1$; $p < 0.001$] and Drug [$F(1,24) = 6.13$; $p < 0.05$], but no Drug × Pump interaction (Fig. 2A, inset).

There was no Pump × Drug interaction in the two-way ANOVA. A separate factorial ANOVA for the 12 h time-point, when 1 mg/kg LY379268 was administered, revealed a significant main effect of Pump [$F(1,24) = 22.8$; $p < 0.001$] but no significant main effect of Drug and no Drug × Pump interaction. Post hoc analysis indicated that 1 mg/kg LY379268 tended to elevate thresholds ($p = 0.06$) in saline- but not nicotine-

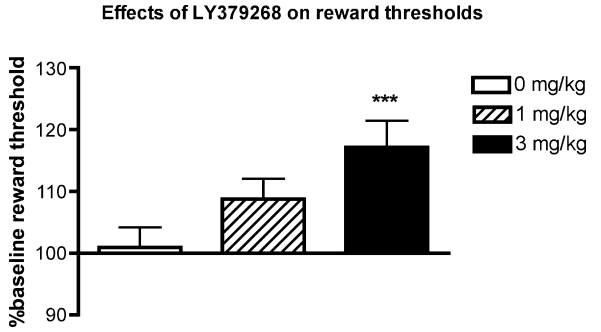

Fig. 1 – LY379268 elevated intracranial self-stimulation thresholds (* $p < 0.001$). Intracranial self-stimulation thresholds are expressed as percent of the values obtained during the preceding baseline session (Session 1) for each subject (mean ± S.E.M., $n = 12$).**

withdrawing rats (Fig. 2B). A separate factorial ANOVA for the 30 h time-point, when 3 mg/kg LY379268 was administered, yielded a significant main effect of Pump [$F(1,24) = 11.43$; $p < 0.01$] and a significant main effect of Drug [$F(1,24) = 10.59$; $p < 0.01$], replicating the threshold-elevating effect of LY379268 observed in the previous study under baseline conditions (see above). Post hoc analysis indicated that the effect of 3 mg/kg LY379268 was significant in previously saline-treated animals ($p < 0.01$) but did not reach significance in previously nicotine-treated animals ($p = 0.09$) (Fig. 2C). There was no Drug × Pump interaction in the factorial ANOVA. Mean ± S.E.M. baseline response latencies before the pump removal were 3.58 ± 0.21 s for the nicotine/vehicle group, 3.37 ± 0.11 s for the nicotine/LY379268 group, 3.31 ± 0.19 s for the saline/vehicle group, and 3.27 ± 0.11 s for the saline/LY379268 group. Twelve hours after pump removal, mean ± S.E.M. percent baseline response latencies were 117 ± 3.4 for the nicotine/vehicle group, 106 ± 3.8 for the nicotine/LY379268 group, 98 ± 2.3 for the saline/vehicle group, and 105 ± 4.1 for the saline/LY379268 group. Thirty hours after pump removal, mean ± S.E.M. percent baseline response latencies were 109 ± 3.1 for the nicotine/vehicle group, 99 ± 3.6 for the nicotine/LY379268 group, 101 ± 2.4 for the saline/vehicle group, and 95 ± 2.7 for the saline/LY379268 group. Response latencies were significantly increased in the nicotine withdrawing group compared to the saline-withdrawing rats [main effect of Pump: $F(1,24) = 9.76$; $p < 0.01$]. In contrast, LY379268 had no effect on response latencies [no significant main effect of Drug and no Drug × Pump interaction].

The total number of somatic signs of nicotine withdrawal was increased 12 h after removal of the minipumps in previously nicotine-treated compared to saline-treated animals (Fig. 3) [main effect of Pump: $F(1,22) = 5.55$; $p < 0.05$]. LY379268 did not change somatic signs associated with nicotine withdrawal (no main effect of Drug and no Drug × Pump interactions). The relatively high dose of 3 mg/kg LY379268 showed behavioral effects in the control subjects including slightly decreased locomotion, writhes, and gasps. These behavoral effects of LY379268 resembled the nicotine abstinence signs and precluded a valid assessment of the effects of 3 mg/kg LY379268 on the nicotine abstinence signs. Thus, somatic signs data are presented only for the 12 h time-point (after 1 mg/kg LY379268).

4. Discussion

Spontaneous nicotine withdrawal elevated ICSS thresholds, indicating a deficit in reward function consistent with previous findings [6]. Elevations in ICSS reward thresholds observed in rats during nicotine withdrawal are considered a model of the affective depression-like aspects of nicotine withdrawal in humans, and more specifically of the symptom of anhedonia [4,6,31]. Contrary to our hypothesis that the mGlu2/3 receptor agonist would aggravate this reward deficit, LY379268 did not significantly affect reward deficits associated with nicotine withdrawal under the conditions used in the present study. A dose of 1 mg/kg LY379268 had no effect in either saline- or nicotine-withdrawing rats. A higher dose of 3 mg/kg LY379268 produced significant reward deficits in saline "withdrawing" animals, but did not significantly affect the reward deficits in nicotine-withdrawing subjects. In addition, 1 mg/kg LY379268 had no effect on somatic signs of nicotine withdrawal, while 3 mg/kg induced non-specific increases in somatic signs even in control subjects. LY379268, at a dose of 1 mg/kg, has been shown previously to reduce reinstatement of cocaine- and heroin-, but not food-, seeking behavior in rats [20–22]. Similarly, 1 mg/kg LY379268 reduced nicotine self-administration but not food-maintained responding [25]. At a dose of 3 mg/kg, LY379268 also reduced reinstatement of food-seeking behavior or food-maintained responding suggesting non-specific motivational or locomotor-suppressing effects of this compound [20,25,32]. Taken together, these results indicate that the mGlu2/3 receptor agonist LY379268 effectively reduces the primary rewarding effects of cocaine [21,33] and nicotine [25], as well as reinstatement of drug-seeking behavior for several drugs of abuse, including nicotine [19–25], at doses that do not exacerbate the reward deficits of nicotine withdrawal. As a limitation of our findings, it should be noted that the 3 mg/kg LY379268 dose was administered after the 1 mg/kg dose in the same animals and at a later time-point during withdrawal. It is possible that administration of 3 mg/kg LY379268 at an earlier time-point would have worsened nicotine withdrawal. In fact 3 mg/kg LY379268 tended to increase reward deficits associated with nicotine withdrawal and elevated thresholds in saline-"withdrawing" subjects, indicating a non-specific effect at higher doses. In addition, we used only one dose of nicotine (3.16 mg/kg/day nicotine base delivered over 10 days). It is possible that LY379268 would affect withdrawal from different doses of nicotine or different durations of nicotine treatment. Finally, nicotine was delivered continuously in our study and not intermittently as in human smokers.

Withdrawal from nicotine is associated with reduced levels of extracellular dopamine and possibly glutamate in limbic areas, such as the nucleus accumbens [14,34,35]. Metabotropic glutamate 2/3 receptors have a modulatory inhibitory role in regulating glutamate [9,36,37] and dopamine [11] transmission. Presynaptic mGlu2/3 autoreceptors are activated during excessive glutamate release and function as a negative feedback mechanism [9]. Accordingly, mGlu2/3 receptor

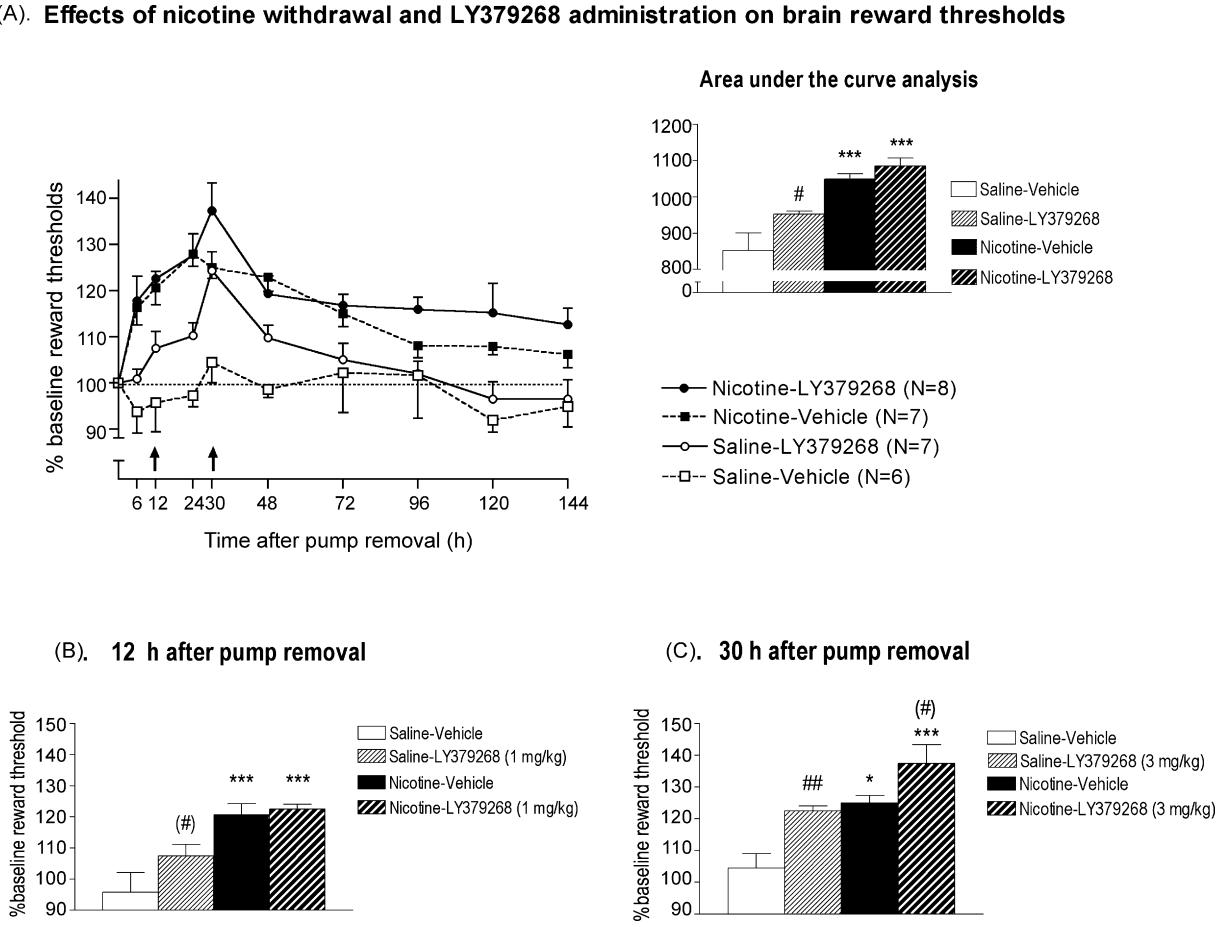

Fig. 2 – Effects of the mGlu2/3 receptor agonist LY379268 on nicotine withdrawal-induced reward threshold elevations. (A) Reward thresholds were measured 6, 12, 24, 30, 48, 72, 96, 120, and 144 h after pump removal. Vehicle or LY379268 (1 mg/kg) was administered 30 min before the 12 h testing time-point, and again vehicle or LY379268 (3 mg/kg) was administered 30 min before the 30 h time-point (arrows). Reward thresholds were significantly elevated after removal of nicotine-delivering pumps but not after removal of saline-releasing pumps. Inset: Area-under-the-curve analysis confirmed that threshold elevations were observed in nicotine-withdrawing rats and saline-withdrawing rats treated with LY379268 compared to saline-vehicle control rats. LY379268 administration in nicotine-withdrawing rats did not significantly elevate thresholds beyond levels seen in nicotine-withdrawing saline-treated rats ($^{***}p < 0.001$, compared to the corresponding saline condition, $^{\#}p < 0.05$ compared to saline-vehicle). (B) At the 12 h time point, 1 mg/kg LY379268 tended to elevate thresholds in saline-withdrawing animals but had no effect in nicotine-withdrawing animals ($^{\#}p = 0.06$). (C) At the 30 h time point, 3 mg/kg LY379268 elevated thresholds significantly in saline-withdrawing ($^{\#\#}p < 0.01$) and non-significantly in nicotine-withdrawing rats ($^{\#}p = 0.09$ compared to nicotine-withdrawing subjects' thresholds). Data are expressed as mean ± S.E.M. percentage change from baseline thresholds (mean of 3 days) prior to removal of nicotine- or saline-containing osmotic minipumps. N = 6–8/group. See Section 3 for details.

stimulation is effective in reversing behaviors associated with increased glutamate release, such as drug self-administration and relapse to drug taking [38,39]. In contrast, the present findings show that LY379268 had no effect on a measure of anhedonia (i.e., diminished interest in the brief electrical stimuli) that is a depression-like aspect of nicotine withdrawal. This lack of effect of LY379268 could be due to a floor effect in glutamate and dopamine levels that are likely to be low during nicotine withdrawal [14], and may not be decreased further by activation of mGlu2/3 receptors. This potential floor effect in glutamate levels appears to result in a ceiling effect in the threshold elevations associated with nicotine withdrawal when LY379268 is administered, because LY379268 did not further elevate thresholds in nicotine-withdrawing subjects as it did in saline-"withdrawing" subjects (present findings). In summary, mGlu2/3 receptor stimulation may not aggravate depressive-like symptoms associated with nicotine withdrawal because neurotransmitter levels are already low and not further affected by stimulation of inhibitory modulatory mGlu2/3 receptors that are located outside of the synaptic cleft [9,40].

Similar to LY379268, the mGlu5 receptor antagonist MPEP also decreases nicotine self-administration [41–43] and reinstatement of nicotine-seeking behavior [44]. However, in contrast to LY379268, previous studies have shown that MPEP significantly elevated brain reward deficits and somatic

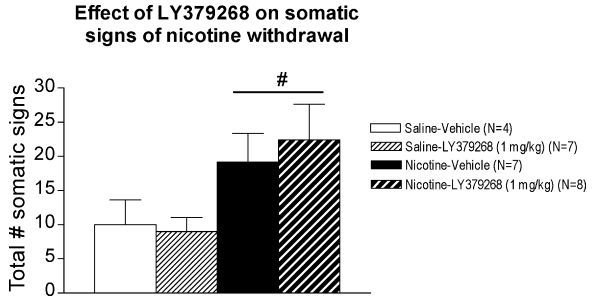

Fig. 3 – Effects of LY379268 (1 mg/kg) on somatic signs of nicotine withdrawal. Twelve hours after removal of nicotine-containing pumps, the total number of somatic signs was significantly increased in animals previously treated with nicotine compared to saline-treated animals [(#) denotes main effect of Pump: $F(1,22) = 5.55$; $p < 0.05$]. LY379268 had no effect on somatic signs of nicotine withdrawal beyond those induced by nicotine withdrawal (no Pump × Drug interaction). See Section 3 for details.

withdrawal signs associated with nicotine withdrawal [43]. Interestingly, the adverse effect of MPEP on nicotine withdrawal was already observed at a low dose of 3 mg/kg, while higher doses were needed to reduce nicotine self-administration or cue-induced reinstatement of nicotine-seeking (6–9 mg/kg or 10 mg/kg, respectively). These findings may indicate that presynaptic mGlu2/3 receptor activation contributes less than postsynaptic mGlu5 receptor antagonism to the anhedonic depression-like aspects of the nicotine-withdrawal state.

The threshold-elevating effects of the mGlu2/3 receptor agonist LY379268 on brain reward function under baseline conditions are consistent with the same effect of the mGlu2/3 receptor agonist LY314582 [45]. Administration of LY314582 systemically or directly into the ventral tegmental area significantly elevated reward thresholds, mimicking the reward deficits of spontaneous nicotine withdrawal, in nicotine-dependent rats at doses that had no effect in control rats [18]. That is, LY314582 precipitated reward deficits in nicotine-dependent rats, similarly to the effects of nicotinic acetylcholine receptor antagonists that precipitate nicotine withdrawal signs [6,30,46,47]. These differences in results are likely explained by the fact that in the study conducted by Kenny et al. [18], the mGlu2/3 receptor agonist was administered while nicotine was delivered by the minipump and thus acting on nicotine-induced enhanced glutamate transmission, while in the present study the mGlu2/3 agonist was administered during a likely hypoglutamatergic state with no nicotine on board at the time of the mGlu2/3 agonist administration. There are also subtle differences in the pharmacodynamic profiles of the two mGlu2/3 receptor agonists LY314582 and LY379268. LY314582 is a racemic mixture of LY354740 [10]. Both, LY354740 and LY379268 are highly selective for mGlu2/3 receptors compared to NMDA, AMPA, or kainate receptors [10]. However, LY379268 is more potent than LY354740 and, interestingly, possesses a significantly different mGlu2/mGlu3 receptor selectivity ratio compared to LY354740 (K_i ratio for mGlu2/mGlu3 = 2.4 and 0.8, respectively) [10]. Thus, LY379268 is more mGlu3-selective than LY354740. Because of these differences, it is possible that LY354740 or other mGlu2/3 receptor agonists may have different effects on nicotine withdrawal than LY379268.

In conclusion, the mGlu2/3 receptor agonist LY379268 induced reward deficits under baseline conditions but did not exacerbate the brain reward deficits or the somatic signs associated with early nicotine withdrawal in rats. Previous findings indicated that this compound decreases nicotine self-administration and cue-induced reinstatement of nicotine-seeking [25]. Thus, similar mGlu2/3 receptor agonists may potentially decrease nicotine intake and relapse to tobacco smoking without adversely affecting somatic and affective symptoms of nicotine withdrawal.

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