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Blockade of CRF1 receptors in the central nucleus of the amygdala attenuates the dysphoria associated with nicotine withdrawal in rats

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Abstract

The majority of smokers relapse during the acute withdrawal phase when withdrawal symptoms are most severe. The goal of the present studies was to investigate the role of corticotropin-releasing factor (CRF) and noradrenergic transmission in the central nucleus of the amygdala (CeA) in the dysphoria associated with smoking cessation. It was investigated if blockade of CRF1 receptors, blockade of α 1-adrenergic receptors, or stimulation of α 2-adrenergic receptors in the CeA diminishes the deficit in brain reward function associated with nicotine withdrawal in rats. Nicotine dependence was induced by implanting minipumps that delivered a nicotine solution. Withdrawal was precipitated with the nicotinic acetylcholine receptor antagonist mecamylamine. A discrete-trial intracranial self-stimulation procedure was used to assess the negative affective aspects of nicotine withdrawal. Elevations in brain reward thresholds are indicative of a deficit in brain reward function. In all the experiments, mecamylamine elevated the brain reward thresholds of the rats chronically treated with nicotine and did not affect the brain reward thresholds of the saline-treated control rats. Intra-CeA administration of the CRF1 receptor antagonist R278995/CRA0450 completely prevented the mecamylamine-induced elevations in brain reward thresholds in the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated control rats. R278995/CRA0450 has also been shown to block sigma-1 receptors but there is no evidence that this could affect negative mood states. Intra-CeA administration of the α 1-adrenergic receptor antagonist prazosin or the α 2-adrenergic receptor agonist clonidine did not affect the brain reward thresholds of the nicotine or saline-treated rats. These studies suggest that CRF1 receptor antagonists may diminish the dysphoria associated with smoking cessation by blocking CRF1 receptors in the CeA.

Keywords

Nicotine; CRF; norepinephrine; central amygdala; withdrawal; ICSS; rats

1. Introduction

Tobacco addiction is a chronic disorder that is characterized by compulsive smoking and relapse after periods of abstinence (American Psychiatric Association, 2000). The positive reinforcing effects of cigarettes have been suggested to play a pivotal role in the initiation of smoking (Chen et al., 2003; Finkenauer et al., 2009). The positive reinforcing effects of

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smoking include mild euphoria, relaxation, and improved cognitive functioning (Ague, 1973; Benowitz, 1988; Wesnes and Warburton, 1983). Smoking induces adaptations in the brain that may cause the negative mood state, increased anxiety, and impaired cognitive function associated with smoking cessation (Bruijnzeel, 2009; Bruijnzeel and Gold, 2005; Hughes et al., 1991). The negative affective symptoms associated with smoking cessation have been suggested to play an important role in relapse to smoking (Koob and Le Moal, 2005).

Corticotropin-releasing factor (CRF) and norepinephrine play a pivotal role in the regulation of mood states. CRF levels are elevated in depressed patients and treatment with antidepressant drugs improves mood states and decreases brain CRF levels (De Bellis et al., 1993; Nemeroff et al., 1984). Furthermore, chronic treatment with noradrenergic reuptake inhibitors improves mood states in depressed patients (Nelson, 1999). More recent studies have provided evidence for a role of CRF and norepinephrine in drug addiction. The administration of the non-specific CRF1/CRF2 receptor antagonist D-Phe CRF(12–41) and the CRF1 receptor antagonist R278995/CRA0450 into the lateral ventricles attenuates the negative mood state associated with nicotine withdrawal (Bruijnzeel et al., 2009; Bruijnzeel et al., 2007). Blockade of CRF1 receptors also attenuates stress-induced reinstatement of nicotine seeking and increased nicotine intake after a period of abstinence (Bruijnzeel et al., 2009; George et al., 2007). Numerous studies have provided evidence for a role of noradrenergic transmission in drug addiction. The α 1-adrenergic receptor antagonist prazosin attenuates the negative mood state associated with nicotine withdrawal in rats (Bruijnzeel et al., 2010). Prazosin also reduces heroin and cocaine self-administration in rats with extended access (6–12 hours/day) to these drugs (Greenwell et al., 2009; Wee et al., 2008). The α 2-adrenergic receptor agonist clonidine attenuates the morphine withdrawal-induced decrease in operant responding for food and prevents naloxone-induced conditioned place aversion in morphine dependent animals (Kosten, 1994; Sparber and Meyer, 1978). Clonidine also improves smoking cessation rates (Glassman et al., 1988; Gourlay et al., 2004) and attenuates opioid withdrawal symptoms in humans (Gold et al., 1978).

Although the aforementioned studies indicate that increased CRF and noradrenergic transmission plays a role in drug withdrawal, very few studies have investigated the role of CRF and noradrenergic transmission in the central nucleus of the amygdala (CeA) in nicotine dependence. The present studies investigated the effect of the CRF1 receptor antagonist R278995/CRA0450 (K_i CRF1 = 53.2 nM; CRF2 >10,000 nM), the α 1-adrenergic receptor antagonist prazosin (IC_{50} α 1 = 0.6 nM, α 2 = 5,000 nM), and the α 2-adrenergic receptor agonist clonidine (K_i α 1 = 713 nM, α 2 = 3.2 nM) in the CeA on precipitated nicotine withdrawal (Chaki et al., 2004; van Meel et al., 1981; Virtanen et al., 1988). Both prazosin and clonidine inhibit noradrenergic transmission in the brain; prazosin by blocking postsynaptic α 1-adrenergic receptors and clonidine by stimulating presynaptic α 2-adrenergic receptors. In a separate experiment, R278995/CRA0450 was administered into the basolateral nucleus of the amygdala (BLA) before precipitating nicotine withdrawal. This control experiment was conducted to rule out the possibility that R278995/CRA0450 affected nicotine withdrawal by blocking CRF1 receptors in brain sites in close proximity to the CeA. The negative mood state associated with nicotine withdrawal was investigated using a discrete-trial intracranial self-stimulation (ICSS) procedure. Previous studies have shown that this ICSS procedure provides a quantitative measure of the emotional aspects of drug withdrawal (Bruijnzeel et al., 2006; Schulteis et al., 1995; Wise and Munn, 1995).

2. Methods

2.1. Subjects

Male Wistar rats (Charles River, Raleigh, NC, USA) weighing 250–300 gram at the beginning of the experiments were used. Animals were group-housed (two per cage) in a temperature and humidity-controlled vivarium and maintained on a 12-hour light–dark cycle (lights off at 8 AM). All testing occurred at the beginning of the dark cycle. Food and water were available ad libitum in the home cages. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the University of Florida Institutional Animal Care and Use Committee (IACUC).

2.2. Drugs

Nicotine hydrogen tartrate salt, mecamylamine hydrochloride, clonidine hydrochloride, prazosin hydrochloride, and pentobarbital sodium salt were purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA). R278995/CRA0450 (1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1,2,3,6-tetrahydropyridine-4-carboxamide benzenesulfonate) was synthesized by Taisho Pharmaceutical Co. (Saitama, Japan). Nicotine, mecamylamine, and pentobarbital were dissolved in saline (0.9 % sodium chloride). R278995/CRA0450, clonidine, and prazosin were dissolved in distilled water. Prazosin was dissolved by the application of heat. Mecamylamine was administered subcutaneously (sc) in a volume of 1 ml/kg body weight and pentobarbital was administered intraperitoneally (ip) in a volume of 2 ml. Drug doses refer to the salt form.

2.3. Cannulae and electrode implantations

At the beginning of the intracranial surgeries, the rats were anesthetized with an isoflurane/oxygen vapor mixture (1–3% isoflurane) and placed in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 3.3 mm below the interaural line (flat skull). The rats were prepared stainless steel cannulae (11 mm long, 23 gauge) above the CeA or BLA using flat skull coordinates according to Paxinos and Watson, 1998, and previous studies by our research group (Marcinkiewicz et al., 2009; Yamada and Bruijnzeel, 2011) and others (Campolongo et al., 2009; Funk et al., 2006). Bilateral cannulae were implanted 2.5 mm above the CeA (anterior-posterior [AP] - 2.3, medial lateral [ML] \pm 4.0 mm, dorsal-ventral [DV] - 4.7 from dura) or 1.5 mm above the BLA (AP - 2.8, ML \pm 5.0 mm, DV - 6.5 from skull). For the electrode implantations, the incisor bar was set 5 mm above the interaural line. The rats were prepared with 11 mm long stainless steel bipolar electrodes (model MS303/2 Plastics One, Roanoke, VA, USA) in the medial forebrain bundle at the level of the posterior lateral hypothalamus (AP - 0.5 mm, ML \pm 1.7 mm, DV - 8.3 mm from dura). The electrodes and cannulae were permanently secured to the skull using dental cement anchored with four skull screws. At the end of the surgery, 11 mm removable 30 gauge wire stylets were inserted in the cannulae to maintain patency.

2.4. Osmotic minipump implantations

The rats were prepared with osmotic minipumps (model 2ML4, 28 day pumps, Durect Corporation, Cupertino, CA, USA) filled with either saline or nicotine. The pumps were implanted subcutaneously under isoflurane/oxygen (1–3% isoflurane) anesthesia. The nicotine concentration was adjusted to compensate for differences in body weight and to deliver 9 mg/kg/day of nicotine salt (3.16 mg/kg/day nicotine base). This dose of nicotine does not have a long-term effect on brain reward thresholds or response latencies of rats in the ICSS procedure (Harrison et al., 2001).

2.5. Apparatus

The experimental apparatus consisted of twelve Plexiglas chambers (30.5 × 30 × 17 cm; Med Associates, Georgia, VT, USA), each housed in a sound-attenuating melamine chamber (Med Associates). The operant chambers consisted of a metal grid floor and a metal wheel (5 cm wide) centered on a sidewall. A photobeam detector was attached next to the response wheel and recorded every 90 degrees of rotation. Brain stimulation was delivered using constant current stimulators (Model 1200C, Stimtek, Acton, MA, USA). Subjects were connected to the stimulation circuit through bipolar leads (Plastics One, Roanoke, VA, USA) attached to gold-contact swivel commutators (model SL2C, Plastics One). A computer controlled the stimulation parameters, data collection, and all test session functions.

2.6. Intracranial self-stimulation procedure

Rats were trained on a modified discrete-trial ICSS procedure (Kornetsky and Esposito, 1979), as described previously (Bruijnzeel et al., 2007; Markou and Koob, 1992). The subjects were trained to turn the wheel on a fixed ratio 1 (FR1) schedule of reinforcement. Each quarter turn resulted in the delivery of a 0.5 second train of 0.1 millisecond cathodal square-wave pulses at a frequency of 100 Hz. After the successful acquisition of responding, defined as 100 reinforcements within 10 minutes, the rats were gradually trained on a discrete-trial current-threshold procedure. Each trial began with the delivery of a non-contingent electrical stimulus, followed by a 7.5 second response window within which the animal could respond to receive a second contingent stimulus identical to the initial non-contingent stimulus. A response during this 7.5 second response window was labeled as a positive response and the lack of a response was labeled as a negative response. During a 2-second period immediately after a positive response, additional responses had no scheduled consequences. The inter-trial interval (ITI), which followed either a positive response or the end of the response window, had an average duration of 10 seconds (7.5 – 12.5 seconds). Responses that occurred during the ITI resulted in an additional 12.5 second delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the ITI period was gradually increased until animals performed consistently at standard test parameters. The rats were subsequently tested on the current-threshold procedure in which stimulation intensities 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 was altered systematically between blocks by 5 μ A steps. The initial stimulus intensity was set 40 μ A above the baseline current-threshold for each animal. Each test session typically lasted 30–40 minutes and provided two variables: brain reward thresholds and response latencies. The brain reward threshold for a descending series was defined as the midpoint between stimulation intensities that supported responding (i.e., positive responses on at least two of the three trials), and current intensities that failed to support responding (i.e., positive responses on fewer than two of the three trials for two consecutive blocks of trials). The threshold for an ascending series was defined as the midpoint between stimulation intensities that did not support responding and current intensities that supported responding for two consecutive blocks of trials. Four threshold estimates were recorded and the mean of these values was taken as the brain reward threshold for a specific subject. The response latency was defined as the time interval between the beginning of the non-contingent stimulus and a positive response. The response latency for each test session was defined as the mean response latency on all trials during which a positive response occurred.

2.7. Intracerebral microinjections

The bilateral injections were administered by using 30 gauge stainless steel injectors that extended 2.5 mm (length of injector tip, 13.5 mm) beyond the guide cannulae. The injection volume was 0.5 μ l / side and this was infused over 66 seconds. The infusion speed was regulated by a Harvard Apparatus syringe pump (model 975) and the pump was equipped with 10 μ l syringes (Hamilton, Rena, NE). The syringes were connected to the injectors with Tygon microbore PVC tubing (0.25 mm ID \times 0.76 mm OD). The injectors were left in place for 10 seconds post-injection to allow diffusion from the injector tip. The 11-mm dummy stylets were re-inserted immediately after the injectors were removed.

2.8. Histology

The placement of the injection sites was verified as in previous studies by our research group in which the role of the CeA in nicotine addiction was investigated (Marcinkiewicz et al., 2009; Yamada and Bruijnzeel, 2011). The rats were euthanized with an overdose of sodium pentobarbital (150 mg, ip) and perfused via the ascending aorta with physiological saline (100 ml) followed by a 10% phosphate buffered formalin solution (150 ml). Brains were postfixed for 24 hours and cryoprotected in 10% sucrose in phosphate buffered saline for 48 hours. Sections were cut on a Leica CM3050 S cryostat (coronal sections of 40 μ m at -15 $^{\circ}$ C). The sections were mounted on Fisher Superfrost Plus slides and stained with cresyl violet. The locations of the guide cannulae and injection sites were verified with light microscopy and with reference to a stereotaxic atlas of the rat brain (Paxinos and Watson, 1998). Histological verification indicated that all the injector tracts ended within or at the boundaries of the CeA (-1.88 to -2.56 AP) or the BLA (-2.56 to -3.30 AP).

2.9. Experimental design

The studies investigated the effects of the intra-CeA administration of R278995/CRA0450, clonidine, and prazosin on precipitated nicotine withdrawal in rats. In an additional control experiment, the effect of the administration of R278995/CRA0450 into the BLA on precipitated nicotine withdrawal was investigated. Drug naïve rats were used for all the experiments. After recovery from the cannulae and electrode implantations, the rats were trained on the ICSS procedure. When stable baseline brain reward thresholds were achieved (defined as less than 10% variation within a 5 day period), the rats were prepared with 28-day osmotic minipumps that contained either saline or nicotine dissolved in saline (Exp. 1A, intra-CeA R278995/CRA0450: saline $n = 9$, nicotine $n = 8$; Exp. 1B, intra-BLA R278995/CRA0450: nicotine $n = 6$; Exp. 2, intra-CeA prazosin: saline $n = 10$, nicotine $n = 10$; Exp. 3, intra-CeA clonidine: saline $n = 8$, nicotine $n = 13$). All drugs were administered according to a Latin-square design and separate groups of rats were used for each experiment. Brain reward thresholds and response latencies were assessed daily throughout the experiment between 9:00 AM and 12:00 noon. The nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine (3 mg/kg, sc) was used to precipitate nicotine withdrawal. Mecamylamine injections started at least 6 days after the implantation of the minipumps to allow time for the development of nicotine dependence. The bilateral intra-CeA and intra-BLA infusions (CeA and BLA R278995/CRA0450, 0.005 – 0.5 μ g; CeA Prazosin 0.3 – 1.5 μ g; CeA Clonidine 0.25 – 1 μ g, unilateral doses) were conducted 10 minutes prior to treatment with mecamylamine. The rats were placed in the ICSS test chambers 5 minutes after mecamylamine administration. The minimum time interval between the mecamylamine injections was at least 72 hours to allow the brain reward thresholds to return to baseline levels. The serum elimination half-life of mecamylamine is approximately 1 hour (Debruyne et al., 2003). At the end of the experiments the rats were perfused under pentobarbital anesthesia and the brains were removed for histological verification of the injection sites.

2.10. Statistical analyses

One-way analyses of variance (ANOVA) were used to verify that the ICSS parameters (absolute brain reward thresholds and response latencies) between the saline rats and nicotine rats did not differ prior to the implantation of the minipumps or prior to the onset of the injections with mecamlamine. One way ANOVA's were also used to compare the absolute brain reward thresholds and response latencies between experiments in which drugs were administered into the CeA. For all the experiments, brain reward thresholds and response latencies were expressed as percentages of the values obtained on the day prior to each test day. Percent changes in brain reward thresholds and response latencies were analyzed by using two-way repeated-measures ANOVA's with the dose of R278995/CRA0450, clonidine, or prazosin as the within-subjects factor and pump content (saline or nicotine) as the between-subjects factor. One way ANOVA's were used to compare the effects of mecamlamine on brain reward thresholds and response latencies between experiments in rats treated with vehicle in the CeA. Statistically significant interactions in the ANOVA were followed by the Newman-Keuls post-hoc test.

3. Results

3.1. Experiments 1A and B: Intra-CeA and intra-BLA administration of R278995/CRA0450 and nicotine withdrawal

There were no differences in absolute brain reward thresholds and response latencies between the saline group and the nicotine group on the test-day prior to the minipump implantations or on the test-day prior to the first mecamlamine / R278995/CRA0450 injections (Experiment 1A, Table 1). Mecamlamine elevated the brain reward thresholds of the nicotine-treated rats, 145%, and did not affect the brain reward thresholds of the control rats (Figure 1A, Treatment: $F_{1,15}=43.98$, $P<0.0001$). Mecamlamine did not affect the response latencies of the nicotine-treated rats or the saline-treated rats (Figure 1B, Treatment: $F_{1,15}=0.61$, n.s.). The administration of R278995/CRA0450 into the CeA prevented the mecamlamine-induced elevations in brain reward thresholds in the nicotine dependent rats and did not affect the brain reward thresholds of the rats that were chronically treated with saline (Figure 1A; Dose \times Treatment interaction: $F_{3,45}=3.27$, $P<0.03$). Newman-Keuls post-hoc comparisons indicated that the administration of 0.05 and 0.5 μg of R278995/CRA0450 into the CeA prevented the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. R278995/CRA0450 did not affect the response latencies (Figure 1B; Dose: $F_{3,45}=0.51$, n.s.; Dose \times Treatment interaction: $F_{3,45}=0.49$, n.s.).

In a separate study, the effect of intra-BLA R278995/CRA0450 on nicotine withdrawal was investigated (Experiment 1B). The administration of R278995/CRA0450 into the BLA did not affect the brain reward thresholds (Table 2, Dose: $F_{3,15}=0.43$, n.s.) or the response latencies (Dose: $F_{3,15}=1.58$, n.s.) of the nicotine dependent rats treated with mecamlamine. This suggests that in the first experiment (Experiment 1A), R278995/CRA0450 prevented the elevations in brain reward thresholds by blocking CRF1 receptors in the CeA and not by blocking CRF1 receptors in brain sites in close proximity to the CeA.

3.2. Experiment 2: Intra-CeA administration of prazosin and nicotine withdrawal

There were no differences in absolute brain reward thresholds and response latencies between the saline group and the nicotine group on the test-day before the minipump implantations or on the test-day before the first mecamlamine / prazosin injections (Table 1). Similar to the first experiment, mecamlamine elevated the brain reward thresholds of the rats that were chronically treated with nicotine and did not affect the brain reward thresholds of the saline-treated rats (Figure 2A, Treatment: $F_{1,18}=83.55$, $P<0.0001$).

Mecamylamine did not affect the response latencies of the nicotine-treated rats or the saline-treated rats (Figure 2B, Treatment: $F_{1,18}=0.0009$, n.s.). Intra-CeA administration of prazosin did not affect the brain reward thresholds (Dose: $F_{3,54}=1.55$, n.s.; Dose \times Treatment: $F_{3,54}=0.38$, n.s.) or the response latencies (Dose: $F_{3,54}=1.69$, n.s.; Dose \times Treatment: $F_{3,54}=0.25$, n.s.) of the rats in the saline group or in the nicotine group.

3.3. Experiment 3: Intra-CeA administration of clonidine and nicotine withdrawal

There were no differences in absolute brain reward thresholds between the saline group and the nicotine group on the test-day prior to the minipump implantations or on the test-day prior to the first mecamylamine / clonidine injections (Table 1). The latency of the nicotine group was slightly longer than the latency of the control group on the test-day prior to the minipump implantations ($F_{1,20}=4.44$, $P<0.049$). However, there was no difference between the latency of the nicotine group and the saline-treated control group on the test-day prior to the first mecamylamine / clonidine injections. The administration of mecamylamine led to an elevation in the brain reward thresholds of the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated rats (Figure 3A, Treatment: $F_{1,19}=34.85$, $P<0.0001$). Mecamylamine did not affect the response latencies of the nicotine-treated rats or the saline-treated rats (Figure 3B, Treatment: $F_{1,19}=0.006$, n.s.). The administration of clonidine into the CeA did not have an effect on the brain reward thresholds (Dose: $F_{3,57}=0.53$, n.s.; Dose \times Treatment: $F_{3,57}=0.88$, n.s.) or the response latencies (Dose: $F_{3,57}=2.17$, n.s.; Dose \times Treatment: $F_{3,57}=4.52$, n.s.) of the saline-treated rats or the nicotine-treated rats.

Additional analyses were conducted to rule out the possibility that some of the differences in the effects of the drugs (intra-CeA: R278995/CRA0450, prazosin, and clonidine) were due to baseline differences between the experiments. There were no baseline differences in absolute brain reward thresholds (Experiment: $F_{2,57}=1.03$, n.s.) and response latencies (Experiment: $F_{2,57}=1.96$, n.s.) between the three experiments. In addition, mecamylamine had the same effect on the brain reward thresholds (Nicotine groups, Experiment: $F_{2,30}=0.16$, n.s.; Saline groups, Experiment: $F_{2,26}=0.42$, n.s.) and the response latencies (Nicotine groups, Experiment: $F_{2,30}=0.22$, n.s.; Saline groups, Experiment: $F_{2,26}=0.07$, n.s.) of the control animals (nicotine or saline animals treated with vehicle in the CeA) in all three experiments.

4. Discussion

The aim of the present studies was to investigate the role of CRF and noradrenergic transmission in the CeA in the negative mood state associated with nicotine withdrawal in rats. In all the experiments, the nAChR antagonist mecamylamine elevated the brain reward thresholds of the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated control rats. Intra-CeA administration of the CRF1 receptor antagonist R278995/CRA0450 prevented the elevations in brain reward thresholds associated with nicotine withdrawal. R278995/CRA0450 did not affect the response latencies and therefore it is unlikely that this drug induced sedative effects or motor impairments. Pretreatment with the α_1 -adrenergic receptor antagonist prazosin and the α_2 -adrenergic receptor agonist clonidine in the CeA did not affect the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. Taken together, these findings would suggest that CRF transmission, but not noradrenergic transmission, in the CeA plays a critical role in the negative mood state associated with tobacco smoking cessation.

The lowest intra-CeA dose of R278995/CRA0450 that prevented the elevations in brain reward thresholds associated with nicotine withdrawal was 0.05 $\mu\text{g}/\text{side}$ (0.1 μg total bilateral dose). The total bilateral dose in the present study is 100 times lower than the

lowest icv dose of R278995/CRA0450, 10 μ g, that prevents the elevations in brain reward thresholds associated with nicotine withdrawal (Bruijnzeel et al., 2009). Therefore, the amount of R278995/CRA0450 that was infused into the CeA would not have prevented the elevations in brain reward thresholds when administered into the lateral ventricles. This excludes the possibility that R278995/CRA0450 diffused into the lateral ventricles and attenuated nicotine withdrawal by acting upon distant brain sites. We also investigated the effect of the administration of R278995/CRA0450 into a brain site in close proximity to the CeA, namely the BLA. The administration of R278995/CRA0450 into the BLA did not affect the brain reward thresholds of nicotine withdrawing rats. This suggests that in the first experiment, R278995/CRA0450 mediated its effects on nicotine withdrawal by blocking CRF1 receptors in the CeA and not by blocking CRF1 receptors in brain sites in close proximity to the CeA such as the BLA.

The administration of a nAChR antagonist to nicotine dependent animals leads to elevations in brain reward thresholds in the ICSS procedure and an increased release of CRF in the CeA (Epping-Jordan et al., 1998; George et al., 2007). The data presented here indicate that blockade of CRF1 receptors in the CeA prevents the elevation in brain reward thresholds associated with nicotine withdrawal. This study extends and corroborates a previous study that showed that the administration of the nonspecific CRF1/CRF2 receptor antagonist D-Phe CRF(12–41) into the CeA prevents the elevations in brain reward thresholds associated with nicotine withdrawal (Marcinkiewicz et al., 2009). The icv administration of the CRF2 receptor antagonist astressin-2B does not affect the elevations in brain reward thresholds associated with precipitated nicotine withdrawal (Bruijnzeel et al., 2009). Therefore these studies strongly suggest that precipitated nicotine withdrawal leads to an increased release of CRF in the CeA which induces a negative mood state via the activation of CRF1, but not CRF2, receptors in this brain site. The negative emotional state associated with nicotine withdrawal may play a role in the development and maintenance of a tobacco addiction in humans. George et al. (2007) showed that the self-administration of nicotine in rats is increased after a period of abstinence (i.e., nicotine-deprivation effect). This deprivation effect is prevented by pretreatment with a specific CRF1 receptor antagonist (George et al., 2007). CRF1 receptor antagonists also prevent the dysphoria and anxiety-like behavior associated with nicotine withdrawal (Bruijnzeel et al., 2009; George et al., 2007). Therefore, these findings suggest that after a period of abstinence, rats may increase their nicotine intake to diminish the CRF-induced dysphoria and anxiety-like behavior.

Pharmacological evidence suggests that the CRF1 receptor antagonist R278995/CRA0450 also blocks sigma-1 receptors (Chaki et al., 2004). Although sigma-1 receptors have been detected in the amygdala, it is unlikely that R278995/CRA0450 prevented the nicotine withdrawal-induced elevations in brain reward thresholds by blocking these receptors (Alonso et al., 2000). Animal studies suggest that stimulation of sigma-1 receptors has antidepressant and anxiolytic-like effects (Kamei et al., 1996; Ukai et al., 1998; Wang et al., 2007). In contrast, blockade of sigma-1 receptors does not affect the behavior of rats and mice in a wide variety of anxiety and depression tests (Chaki et al., 2004; Noda et al., 2000; Ukai et al., 1998).

The present study showed that blockade of α 1-adrenergic receptors and stimulation of α 2-adrenergic receptors in the CeA does not affect the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. It is unlikely that the lack of effect of prazosin or clonidine on nicotine withdrawal was due to the absence of α 1 or α 2-adrenergic receptors in the CeA. Previous studies have shown that α 1 and α 2-adrenergic receptors are expressed in this brain site (Rainbow and Biegon, 1983; Young, III and Kuhar, 1980). It is also unlikely that the doses of clonidine or prazosin were too low. Previous studies by our group and others have shown that lower doses than those used in the present studies affect

the behavior of rats (Ferry et al., 1999; Yamada and Bruijnzeel, 2011). The highest dose of clonidine in the present study was 1 µg/side. In a previous study, we reported that the bilateral administration of the same dose of clonidine into the CeA attenuates stress-induced reinstatement of nicotine-seeking behavior in rats (Yamada and Bruijnzeel, 2011). At this point, we are not aware of any studies that investigated the behavioral effects of the administration of prazosin in the CeA. However, one study investigated the effects of the administration of prazosin into the BLA in a learning and memory test. It was shown that the administration of 0.1 – 1 µg/side of prazosin produces deficits in shock-induced avoidance learning (Ferry et al., 1999). These doses fall in the same dose-range as the prazosin doses, 0.3 – 1.5 µg/side, which were used in the present nicotine withdrawal study.

In a previous study we reported that the systemic administration of the α 1-adrenergic receptor antagonist prazosin attenuates the deficit in brain reward function associated with precipitated nicotine withdrawal (Bruijnzeel et al., 2010). One of the aims of the present experiments was to investigate if the effects of prazosin on nicotine withdrawal were mediated by blocking α 1-adrenergic receptors in the CeA. This study demonstrated that the effects of systemically administered prazosin on nicotine withdrawal were not mediated by α 1-adrenergic receptors in the CeA. Future studies will explore the role of α 1-adrenergic receptors in other brain sites in nicotine withdrawal. A binding study with the selective α 1-adrenergic receptor agonist HEAT has shown that high levels of α 1-adrenergic receptors are expressed in the bed nucleus of the stria terminalis (Jones et al., 1985). In addition, noradrenergic projections from the A1 and A2 noradrenergic cell groups in the brainstem to the BNST have been implicated in opioid withdrawal-induced place aversion (Delfs et al., 2000). Therefore, it might be possible that α 1-adrenergic receptors in the BNST play a role in nicotine withdrawal.

In conclusion, the present findings indicate that the activation of CRF1 receptors in the CeA mediates the elevations in brain reward thresholds associated with nicotine withdrawal. This would suggest that CRF1 receptor antagonists may attenuate the dysphoria associated with smoking cessation by blocking CRF1 receptors in the CeA. The dysphoria associated with smoking cessation increases craving for cigarettes and contributes to relapse to smoking (Doherty et al., 1995; West et al., 1989). Therefore, CRF1 receptor antagonists may improve smoking cessation rates by attenuating the negative mood state associated with smoking cessation.

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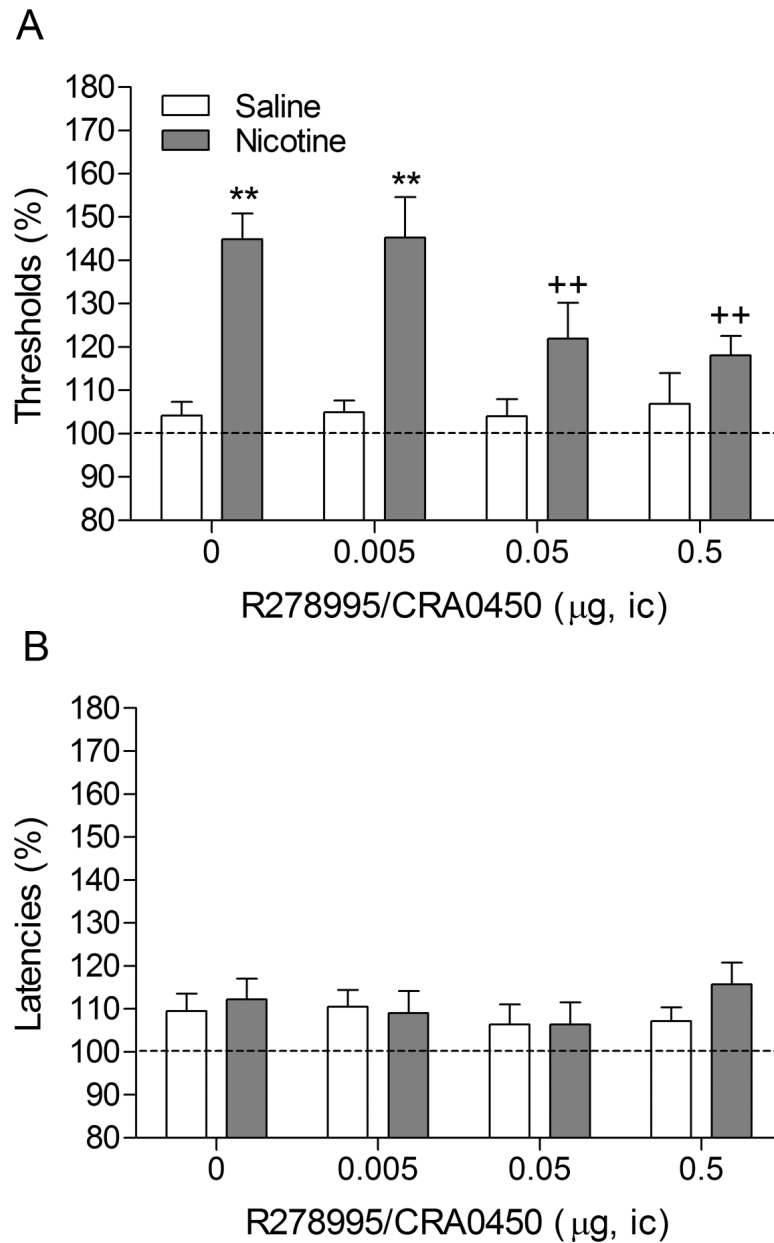


Fig. 1. Effect of intra-CeA administration of the CRF1 receptor antagonist R278995/CRA0450 on the elevations in ICSS thresholds associated with precipitated nicotine withdrawal (A; saline $n = 9$, nicotine $n = 8$). Effect of intra-CeA R278995/CRA0450 on the response latencies of rats chronically treated with saline or nicotine and acutely treated with mecamylamine (B; saline $n = 9$, nicotine $n = 8$). ICSS thresholds and response latencies are expressed as a percentage of the pre-test day values. R278995/CRA0450 was administered bilaterally and the figures depict the unilateral dose. Asterisks (** $P < 0.01$) indicate elevations in ICSS thresholds compared to those of the corresponding saline-treated control group. Plus signs (++) $P < 0.01$) indicate lower ICSS thresholds compared to those of rats chronically treated with nicotine and acutely treated with mecamylamine and vehicle (0 µg of R278995/CRA0450). Abbreviation: ic, intracranial.

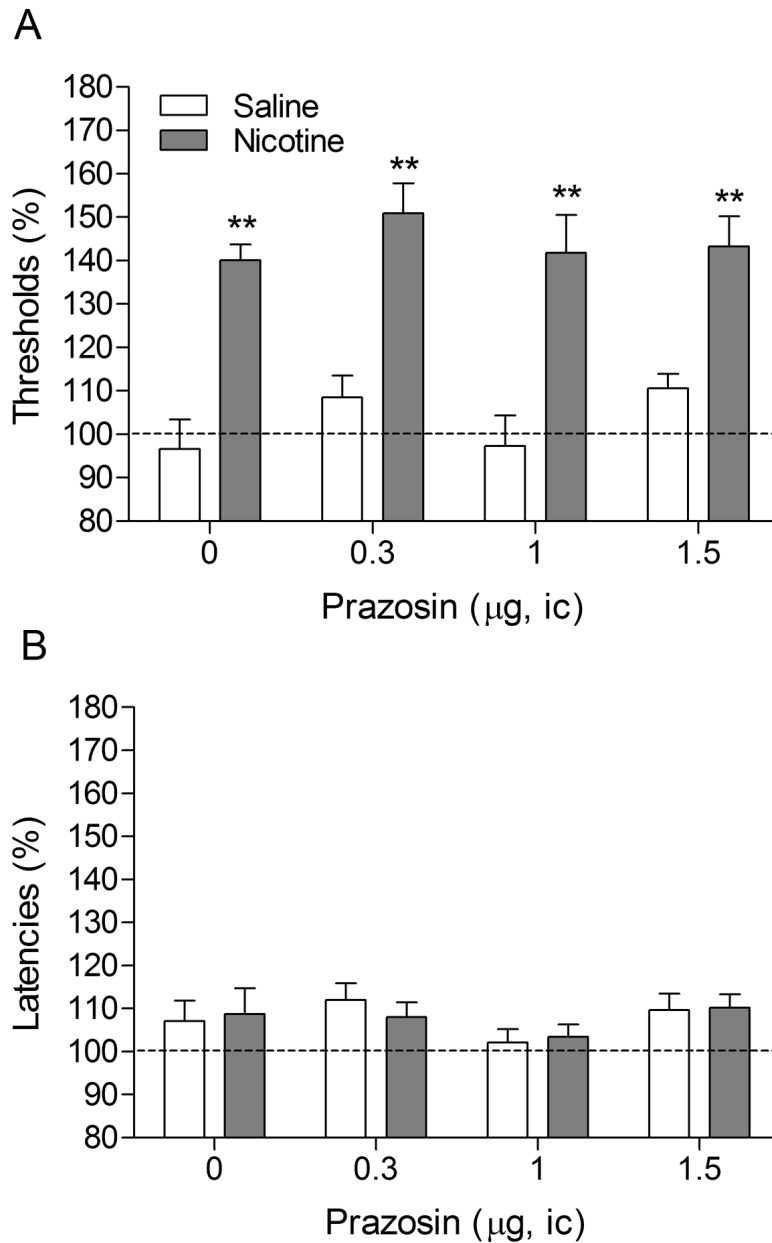


Fig. 2. Effect of intra-CeA administration of the $\alpha 1$ -adrenergic receptor antagonist prazosin on the elevations in ICSS thresholds associated with precipitated nicotine withdrawal (A; saline, $n = 10$; nicotine, $n = 10$). Effect of intra-CeA prazosin on the response latencies of rats chronically treated with saline or nicotine and acutely treated with mecamylamine (B; saline $n = 10$, nicotine $n = 10$). ICSS thresholds and response latencies are expressed as a percentage of the pre-test day values. Prazosin was administered bilaterally and the figures depict the unilateral dose. Abbreviation: ic, intracranial.

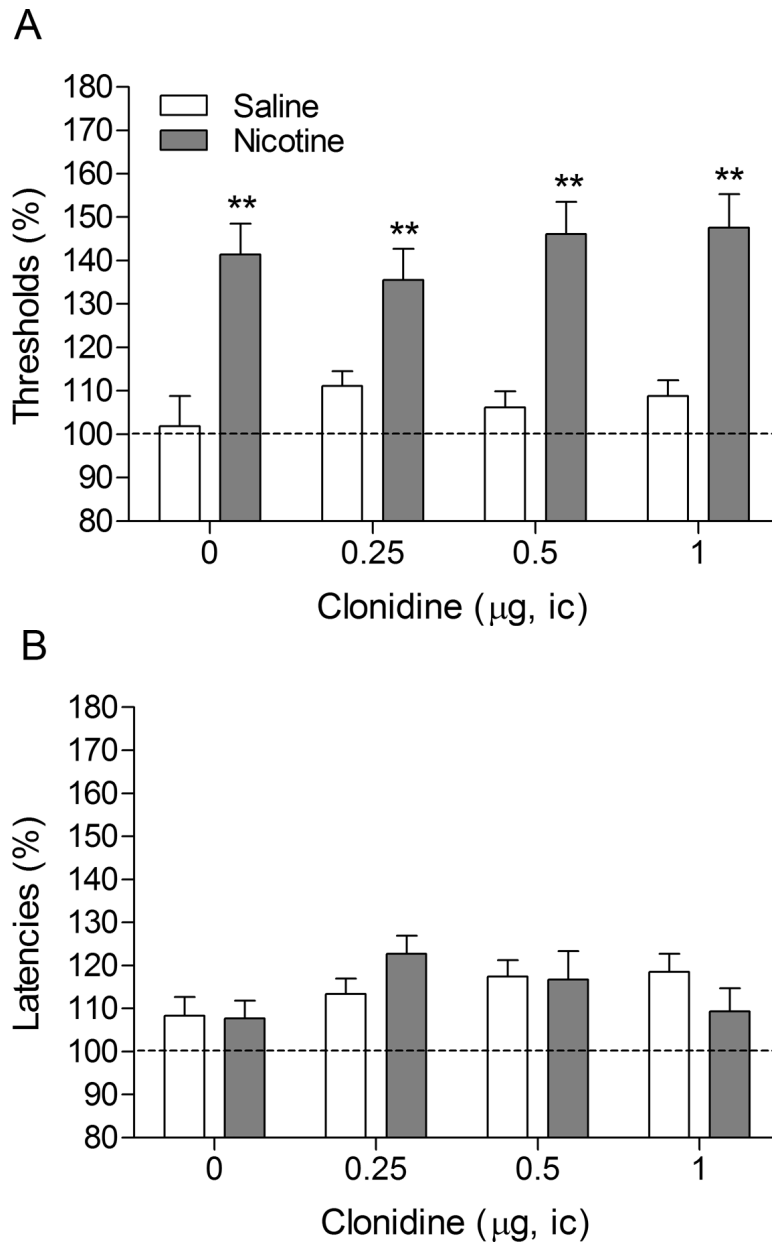


Fig. 3. Effect of intra-CeA administration of the α_2 -adrenergic receptor agonist clonidine on elevations in ICSS thresholds associated with precipitated nicotine withdrawal (A; saline, $n = 8$; nicotine, $n = 13$). Effect of intra-CeA clonidine on the response latencies of rats chronically treated with saline or nicotine and acutely treated with mecamylamine (B; saline $n = 8$, nicotine $n = 13$). ICSS thresholds and response latencies are expressed as a percentage of the pre-test day values. Clonidine was administered bilaterally and the figures depict the unilateral dose. Abbreviation: ic, intracranial.

Table 1

Absolute baseline brain reward thresholds and response latencies.

	Thresholds (μ A)		Latencies (s)	
	Saline	Nicotine	Saline	Nicotine
Exp. 1A R278995/CRA0450-CeA (saline, n = 9; nicotine, n = 8)				
Prior pump implantation	123.3 \pm 14.5	119.0 \pm 13.2	3.1 \pm 0.1	3.0 \pm 0.2
Prior first injection	113.8 \pm 15.3	108.8 \pm 13.4	3.2 \pm 0.2	3.0 \pm 0.2
Exp. 1B R278995/CRA0450-BLA (nicotine, n = 6)				
Prior pump implantation	N/A	85.8 \pm 9.3	N/A	3.5 \pm 0.1
Prior first injection	N/A	82.7 \pm 11.3	N/A	2.9 \pm 0.1
Exp. 2 Prazosin-CeA (saline, n = 10; nicotine, n = 10)				
Prior pump implantation	96.3 \pm 7.1	88.8 \pm 8.5	3.3 \pm 0.1	3.3 \pm 0.1
Prior first injection	100.2 \pm 6.6	94.4 \pm 8.9	3.5 \pm 0.1	3.4 \pm 0.1
Exp. 3 Clonidine-CeA (saline, n = 8; nicotine, n = 13)				
Prior pump implantation	100.7 \pm 7.7	112.5 \pm 10.6	3.1 \pm 0.1	3.6 \pm 0.2*
Prior first injection	101.1 \pm 8.1	115.7 \pm 11.4	3.2 \pm 0.1	3.5 \pm 0.2

Prior refers to the test-day immediately before the implantation of the osmotic minipumps or the start of the mecamylamine injections. Asterisk ($P < 0.05$) indicates that the latency of the nicotine group is longer than the latency of the saline group. Abbreviations: CeA, central nucleus of the amygdala; BLA, basolateral nucleus of the amygdala.

Table 2

Intra-BLA administration of the CRF1 receptor antagonist R278995/CRA0450 does not affect the ICSS thresholds and response latencies of nicotine withdrawing rats.

R278995/CRA0450		
Dose (μg, ic)	Thresholds	Latencies
0	145.6 \pm 5.9	120.1 \pm 3.1
0.005	139.2 \pm 8.4	107.5 \pm 4.1
0.05	146.4 \pm 10.2	118.8 \pm 7.5
0.5	137.4 \pm 9.4	107.9 \pm 5.6

ICSS thresholds and response latencies are expressed as a percentage of the pre-test day values (n = 6). R278995/CRA0450 was administered bilaterally and the unilateral dose is depicted in the table. Abbreviation: ic, intracranial.