

# Corticotropin-Releasing Factor-1 Receptor Activation Mediates Nicotine Withdrawal-Induced Deficit in Brain Reward Function and Stress-Induced Relapse

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**Background:** Tobacco addiction is a chronic brain disorder that is characterized by a negative affective state upon smoking cessation and relapse after periods of abstinence. Previous research has shown that blockade of corticotropin-releasing factor (CRF) receptors with a nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonist prevents the deficit in brain reward function associated with nicotine withdrawal and stress-induced reinstatement of extinguished nicotine-seeking in rats. The aim of these studies was to investigate the role of CRF<sub>1</sub> and CRF<sub>2</sub> receptors in the deficit in brain reward function associated with precipitated nicotine withdrawal and stress-induced reinstatement of nicotine-seeking.

**Methods:** The intracranial self-stimulation (ICSS) procedure was used to assess the negative affective state of nicotine withdrawal. Elevations in brain reward thresholds are indicative of a deficit in brain reward function. Stress-induced reinstatement of nicotine-seeking was investigated in animals in which responding for intravenously infused nicotine was extinguished by substituting saline for nicotine.

**Results:** In the ICSS experiments, the nicotinic receptor antagonist mecamylamine elevated the brain reward thresholds of the nicotine-dependent rats but not those of the control rats. The CRF<sub>1</sub> receptor antagonist R278995/CRA0450 but not the CRF<sub>2</sub> receptor antagonist astressin-2B prevented the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. Furthermore, R278995/CRA0450 but not astressin-2B prevented stress-induced reinstatement of extinguished nicotine-seeking. Neither R278995/CRA0450 nor astressin-2B affected operant responding for chocolate-flavored food pellets.

**Conclusions:** These studies indicate that CRF<sub>1</sub> receptors but not CRF<sub>2</sub> receptors play an important role in the anhedonic-state associated with acute nicotine withdrawal and stress-induced reinstatement of nicotine-seeking.

**Key Words:** Anhedonia, mecamylamine, nicotine, rats, relapse, withdrawal

Tobacco addiction is a chronic disorder that is characterized by loss of control over smoking, withdrawal symptoms upon smoking cessation, and relapse after periods of abstinence (1). Abrupt cessation of smoking typically mediates negative affective symptoms such as depressed mood and anxiety. It has been hypothesized that the negative affective aspects of tobacco withdrawal provide powerful motivation for the continuation of smoking (2,3). After the acute withdrawal phase, exposure to stressors increases the likelihood of relapse to smoking (4,5). Pharmacotherapies that diminish the negative affective state of tobacco withdrawal and reduce the risk for stress-induced relapse might improve long-term smoking cessation rates.

Animal models have been developed to study the negative mood state associated with drug withdrawal and stress-induced relapse. Discontinuation of cocaine, amphetamine, alcohol, fentanyl, and nicotine administration elevates brain reward thresholds in a discrete-trial intracranial self-stimulation (ICSS) procedure (6–10). Elevations in brain reward thresholds are interpreted as a deficit in brain reward function as higher current intensities are required to maintain responding for rewarding electrical stimuli (11). After the acute withdrawal phase, stressors

can increase the risk for relapse (4,5). Footshocks have been shown to induce the reinstatement of extinguished cocaine-, heroin-, nicotine-, and alcohol-seeking in rats (12–15). Extensive evidence points toward a role for the neuropeptide corticotropin-releasing factor (CRF) in stress-induced reinstatement of drug seeking. Blockade of CRF receptors with nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonists or small-molecule CRF<sub>1</sub> receptor antagonists prevents stress-induced reinstatement of alcohol-, cocaine-, and heroin seeking (16–20).

In previous studies we demonstrated that the nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonist D-Phe CRF<sub>(12-41)</sub> prevents the deficit in brain reward function associated with precipitated nicotine withdrawal and stress-induced reinstatement of nicotine-seeking (21,22). These studies did not indicate whether acute nicotine withdrawal or stress-induced reinstatement of nicotine-seeking was mediated via the activation of CRF<sub>1</sub> or CRF<sub>2</sub> receptors. Substantial evidence suggests that CRF<sub>1</sub> receptors play a role in alcohol and nicotine withdrawal-induced anxiety-like behavior and alcohol and nicotine intake in dependent animals (23–25). Conflicting findings have been reported with regard to the role of CRF<sub>2</sub> receptors in negative emotional states and drug withdrawal (26,27). Therefore, it is not known whether blockade of CRF<sub>2</sub> receptors contributes to the anti-stress effects of nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonists such as D-Phe CRF<sub>(12-41)</sub>. During the last decade, several small-molecule CRF<sub>1</sub> receptor antagonists have been developed that can cross the blood brain barrier and display efficacy in clinical trials for anxiety and depression (28). Therefore, it is important to know whether selective CRF<sub>1</sub> receptor antagonists can diminish the negative mood state associated with smoking cessation and prevent stress-induced relapse. In the present studies the selective CRF<sub>1</sub> receptor antagonist R278995/CRA0450 and the selective CRF<sub>2</sub> receptor antagonist astressin-2B were used to investigate the role

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of CRF<sub>1</sub> and CRF<sub>2</sub> receptors in the negative affective state of nicotine withdrawal and stress-induced reinstatement of drug-seeking (29,30). The negative affective state of nicotine withdrawal was investigated by using a discrete-trial current-threshold ICSS procedure (31). The role of specific CRF receptors in stress-induced reinstatement of nicotine-seeking was investigated by using a previously established reinstatement procedure (14,22). To investigate whether R278995/CRA0450 or astressin-2B induced a nonspecific impairment in motor function, the effects of these CRF receptor antagonists on food responding was investigated (32).

## Methods and Materials

### Subjects

Male Wistar rats (Charles River, Raleigh, North Carolina) weighing 250–300 g were used. The ICSS rats were group-housed and maintained on a 12-hour light-dark cycle (lights off at 6:00 PM). The intravenous self-administration (IVSA) rats were single-housed and maintained on a 12-hour reversed light-dark cycle (lights on at 6 PM). The ICSS rats received ad libitum food and water. The IVSA rats received ad libitum food and water except during the IVSA period when they were fed 20 g of food immediately after the IVSA sessions. 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.

### Drugs

Nicotine, the nicotinic acetylcholine receptor antagonist mecamylamine (33,34), and pentobarbital were purchased from Sigma-Aldrich (St. Louis, Missouri) and dissolved in sterile saline. The R278995/CRA0450 (1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1,2,3,6-tetrahydropyridine-4-carboxamide benzenesulfonate) was synthesized by Taisho Pharmaceutical Company (Saitama, Japan). Astressin-2B (cyclo(31-34)[D<sup>11</sup>Phe<sup>11</sup>,His<sup>12</sup>,Nle<sup>17</sup>,C<sup>α</sup>MeLeu<sup>13</sup>,39,Glu<sup>31</sup>,Lys<sup>34</sup>Ac-Sau<sup>(8-40)</sup>]) was synthesized at The Salk Institute for Biological Studies (La Jolla, California). The R278995/CRA0450 and astressin-2B were dissolved in distilled water and administered within 1 hour after being dissolved. The astressin-2B solution was kept on ice until being used. The R278995/CRA0450 is a selective CRF<sub>1</sub> receptor antagonist (IC<sub>50</sub> of 53.2 nmol/L for CRF<sub>1</sub> receptor, K<sub>i</sub> of >10,000 nmol/L for CRF<sub>2</sub> receptor), and astressin-2B is a selective CRF<sub>2</sub> receptor antagonist (IC<sub>50</sub> > 500 nmol/L for CRF<sub>1</sub> receptor, IC<sub>50</sub> of 1.3 nmol/L for CRF<sub>2</sub> receptor) (29,30).

### Surgical Procedures

For experiments 1 and 2, the rats were prepared with an 11-mm electrode in the medial forebrain bundle and an 11-mm cannula above the lateral ventricle (21). The rats were anesthetized with an isoflurane/oxygen vapor mixture and placed in a stereotaxic frame with the incisor bar set 3.3 mm below the interaural line (flat skull). The cannulae were implanted above the lateral ventricle with the following flat skull coordinates: anterior posterior (AP) –9 mm, medial lateral (ML) ± 1.4 mm, and dorsal ventral (DV) –3.0 mm from skull (35). The electrodes were implanted in the medial forebrain bundle by using the following coordinates: AP –.5 mm, ML ±1.7 mm, DV –8.3 mm from dura (incisor bar 5 mm above interaural line). For experiments 3 and 4, the rats were prepared with a cannula above the

lateral ventricle and a chronic catheter in the right jugular vein as described previously (22).

### ICSS Procedure

Rats were trained on a modified discrete-trial ICSS procedure (36), as described previously (31). The operant conditioning chambers were housed in sound-attenuating chambers (Med Associates, Georgia, Vermont). The operant conditioning chambers had a 5-cm-wide metal response wheel centered on a sidewall, and a photobeam detector recorded every 90° of rotation. Brain stimulation was delivered by constant current stimulators (Model 1200C, Stimtek, Acton, Massachusetts). The rats were trained on a discrete-trial current-threshold procedure. Each test session provided a brain reward threshold and a response latency. The brain reward threshold was defined as the midpoint between stimulation intensities that supported responding and current intensities that failed to support responding. The response latency was defined as the time interval between the beginning of the noncontingent stimulus and a positive response.

### Nicotine Self-Administration, Extinction, and Stress-Induced Reinstatement

Drug self-administration sessions were conducted as described previously (22). Briefly, rats were trained to respond for food pellets under a fixed-ratio 5, time-out 20-sec (FR5 TO20-s) schedule of reinforcement. After completion of food training the rats were allowed to self-administer nicotine at the .03-mg/kg/infusion (free base) dose. Responding on the active lever resulted in the delivery of a nicotine infusion (.1 mL infused over a 5.6-sec time-period). Responding for nicotine was extinguished by replacing nicotine with saline. Nicotine-seeking was reinstated by the administration of footshocks (eight shocks in a 10-min time-period, .8 mA, 1-sec shocks, mean off period 37 sec) immediately before the self-administration session.

### Intracranial Drug Administration

For intracerebroventricular injections, stainless steel injectors projecting 2.5 mm beyond the guide cannulae were used. All injections were made by gravity induced by raising the 10- $\mu$ L Hamilton syringe. Five  $\mu$ L of solution were administered over a 30–60-sec period, and the injector was left in place for another 30 sec to allow diffusion from the injector tip.

### Statistical Analyses

The ICSS parameters were analyzed by two-way repeated-measures analyses of variance (ANOVA) with the dose of the CRF receptor antagonist as the within-subjects factor and pump content (saline or nicotine) as the between-subjects factor. The self-administration of nicotine over time was analyzed by one-way repeated-measures ANOVA with time as the within-subjects factor. The effect of extinction training on lever pressing was analyzed by one-way repeated-measures ANOVA with time as the within subjects factor. The effect of footshocks on lever pressing was analyzed with a paired sample *t* test. The effect of the CRF receptor antagonists on lever pressing after the administration of footshocks was analyzed by one-way repeated-measures ANOVA with the dose of the antagonist as the within subjects factor. The effect of the CRF receptor antagonists on food responding was analyzed by one-way repeated-measures ANOVA with the dose of the CRF antagonist as the within subjects factor. Statistically significant results in the ANOVAs were followed by the Newman-Keuls post hoc test.

## Experimental Design

**Experiment 1: Effect of R278995/CRA0450 on Precipitated Nicotine Withdrawal.** The rats were trained on the ICSS procedure, and when stable baseline brain reward thresholds were achieved (defined as < 10% variation within a 5-day period), the rats were prepared with 28-day osmotic minipumps containing either saline ( $n = 12$ ) or nicotine ( $n = 14$ , 9 mg/kg/day of nicotine salt, Alzet model 2ML4). The ICSS parameters were assessed daily between 9:00 AM and 12:00 noon. Mecamylamine (3 mg/kg, SC) injections started at least 6 days after the implantation of the minipumps to allow the development of nicotine dependence. The CRF<sub>1</sub> receptor antagonist R278995/CRA0450 (1–20  $\mu$ g, ICV) was administered 15 min before treatment with mecamylamine. The rats were placed in the ICSS test chambers 5 min after mecamylamine administration. To allow the reestablishment of nicotine dependence the minimum time interval between the mecamylamine injections was at least 72 hours.

**Experiment 2: Effect of Astressin-2B on Precipitated Nicotine Withdrawal.** This experiment was the same as experiment 1, with the exception that the CRF<sub>2</sub> receptor antagonist astressin-2B (1–20  $\mu$ g, ICV) was administered to rats treated with nicotine ( $n = 8$ ) or saline ( $n = 8$ ).

**Experiment 3: Effect of R278995/CRA0450 on Stress-Induced Reinstatement of Nicotine-Seeking.** Drug-naïve rats ( $n = 12$ ) were trained to respond for food pellets and then allowed to self-administer nicotine (FR5 TO20-s, .03 mg/kg of nicotine/infusion; free base) for 14 consecutive days. Nicotine-seeking behavior was extinguished by substituting saline for nicotine. Extinction training was considered completed when the average number of infusion was <2. Reinstatement sessions started 1 day after extinction training was completed. The R278995/CRA0450 (5, 20  $\mu$ g, ICV) was administered according to a Latin-square design 15 min before the footshock sessions. There were at least 2 off-days between test days, and on these days the rats were left undisturbed. The design of this study was based on previous studies that showed that repeated footshock sessions or repeated exposure to cues associated with nicotine delivery reliably reinstates extinguished nicotine-seeking (14,22,37). At the end of the experiment the rats were killed with an overdose of pentobarbital (150 mg/kg, IP), and cannulae placement were verified by administering 5  $\mu$ L of a .5% aqueous methyl blue solution at the injection site.

**Experiment 4: Effect of Astressin-2B on Stress-Induced Reinstatement of Nicotine-Seeking.** The design of this experiment was the same as that of experiment 3, with the exception that astressin-2B (0, 5, 20  $\mu$ g, ICV) was administered to the animals ( $n = 16$ ) 15 min before the footshock session.

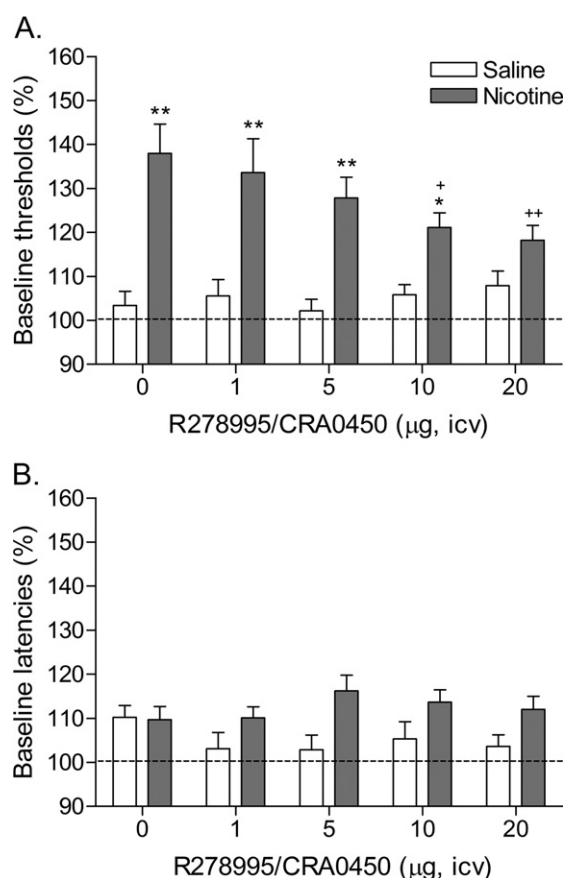
**Experiment 5: Effects of R278995/CRA0450 and Astressin-2B on Responding for Food Pellets.** The rats from experiment 3 were used to investigate the effect of R278995/CRA0450 on food responding ( $n = 11$  [1 rat died before the onset of experiment 5]), and the rats from experiment 4 were used to investigate the effect of astressin-2B on food responding ( $n = 16$ ). First, the rats were allowed to respond for chocolate-flavored food pellets under an FR5 TO20-s schedule of reinforcement. After approximately 1 week, the test sessions with R278995/CRA0450 or astressin-2B were initiated. The R278995/CRA0450 (0, 5, 20  $\mu$ g, ICV) or astressin-2B (0, 5, 20  $\mu$ g, ICV) was administered according to a Latin-square design 15 min before the rats were placed in the operant conditioning chambers. There were at least 2 off-days between the drug days. The animals were allowed to respond for chocolate-flavored food pellets on off-days. The rats were allowed to respond for food 7 days/week,

and all the sessions were 20 min. At the end of the experiment the rats were killed with an overdose of pentobarbital, and cannulae placement were verified.

## Results

### Experiment 1: Effect of R278995/CRA0450 on Precipitated Nicotine Withdrawal

Mean ( $\pm$  SEM) absolute brain reward thresholds before minipump-implantation for saline- and nicotine-treated rats were 101.01  $\pm$  6.53  $\mu$ A and 101.61  $\pm$  8.72  $\mu$ A, respectively. Mean ( $\pm$  SEM) absolute response latencies for saline- and nicotine-treated rats were 3.18  $\pm$  .10 sec and 3.30  $\pm$  .11 sec, respectively. Figure 1 indicates that mecamylamine elevated the brain reward thresholds of the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated rats [Figure 1A; Treatment:  $F(1,24) = 28.491$ ,  $p < .0001$ ]. Pretreatment with R278995/CRA0450 prevented the mecamylamine-induced elevations in brain reward thresholds in the nicotine-treated rats and did



**Figure 1.** Effect of the corticotropin-releasing factor (CRF)<sub>1</sub> receptor antagonist R278995/CRA0450 (saline,  $n = 12$ ; nicotine,  $n = 14$ ) on the elevations in brain reward thresholds associated with mecamylamine-precipitated nicotine withdrawal (A). Effect of R278995/CRA0450 on the response latencies of rats chronically treated with saline ( $n = 12$ ) or nicotine ( $n = 14$ ) and acutely treated with mecamylamine (B). Brain reward thresholds and response latencies are expressed as a percentage of the pre-test day values. Asterisks ( $*p < .05$ ,  $**p < .01$ ) indicate elevations in brain reward thresholds compared with those of the corresponding saline-treated control group. Plus signs ( $+p < .05$ ,  $++p < .01$ ) indicate lower brain reward thresholds compared with those of rats chronically treated with nicotine and acutely treated with mecamylamine and vehicle (0  $\mu$ g of R278995/CRA0450). Data are expressed as means  $\pm$  SEM.

not affect the brain reward thresholds of the saline-treated rats [Dose  $\times$  Treatment interaction:  $F(4,96) = 3.162, p < .017$ ]. Newman-Keuls post hoc comparisons indicated that 20  $\mu\text{g}$  of R278995/CRA0450 attenuated the elevations in brain reward thresholds associated with nicotine withdrawal. Mecamylamine increased the response latencies of the nicotine-treated rats compared with those of the saline-treated rats [Figure 1B; Treatment:  $F(1,24) = 16.381, p < .0005$ ]. The R278995/CRA0450 did not affect the response latencies of the nicotine- or saline-treated rats (Figure 1B).

### Experiment 2: Effect of Astressin-2B on Precipitated Nicotine Withdrawal

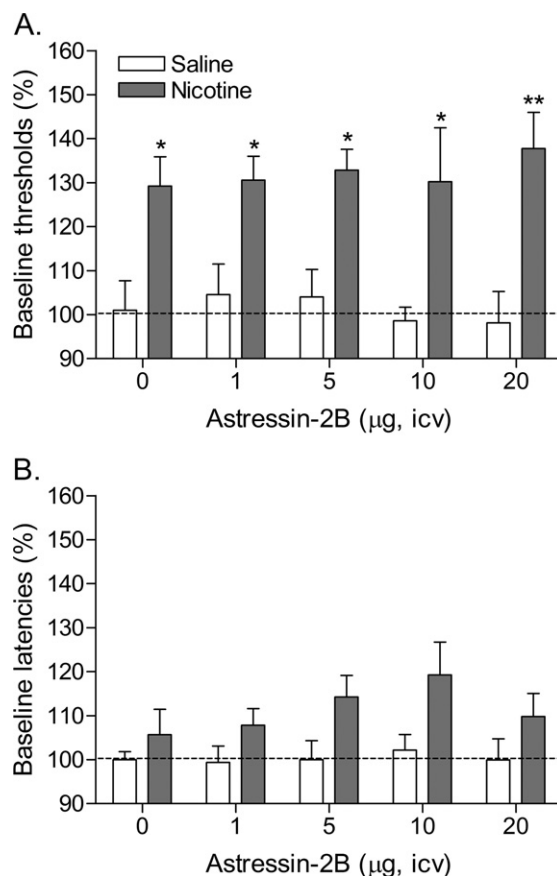
Mean ( $\pm$  SEM) absolute brain reward thresholds before pump-implantation for the saline- and nicotine-treated rats were  $123.07 \pm 3.24 \mu\text{A}$  and  $122.80 \pm 3.13 \mu\text{A}$ , respectively. Mean ( $\pm$  SEM) absolute response latencies for saline- and nicotine-treated rats were  $3.24 \pm .10$  sec and  $3.13 \pm .10$  sec, respectively. Mecamylamine elevated the brain reward thresholds of the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated rats [Figure 2A; Treatment:  $F(1,14) = 29.931, p < .0001$ ]. Pretreatment with astressin-2B did not affect the brain reward thresholds of the nicotine-treated rats or the saline-treated rats (Figure 2A). Mecamylamine increased the response latencies of the nicotine-treated rats compared with those of the saline-treated rats [Figure 2B; Treatment:  $F(1,14) = 15.824, p < .001$ ]. Astressin-2B did not affect the response latencies of the nicotine-treated rats or the saline-treated rats (Figure 2B).

### Experiment 3: Effect of R278995/CRA0450 on Stress-Induced Reinstatement of Nicotine-Seeking

The mean ( $\pm$  SEM) number of infusions of .03 mg/kg of nicotine, responses on the active lever, and responses on the inactive lever during the last day of nicotine self-administration were  $20.50 \pm 2.48$ ,  $103.67 \pm 12.37$ , and  $8.75 \pm 1.86$ , respectively (Figure 3A). After the onset of nicotine self-administration, responding on the active lever initially decreased and then increased [Time:  $F(13,143) = 5.222, p < .0001$ ]. Responding on the inactive lever gradually increased [Time:  $F(13,143) = 3.347, p < .0002$ ]. Substituting saline for nicotine caused a rapid decline in the number of responses on the active lever [Figure 3B;  $F(8,88) = 28.184, p < .0001$ ] and did not affect the number of responses on the inactive lever. The extinction criterion ( $< 2$  infusions) was met 9 days after the onset of extinction training. The administration of footshocks increased the number of responses on the active lever [Figure 3B; last day of extinction vs. footshocks vehicle condition;  $t(11) = 3.59, p < .004$ ] and did not affect the number of responses on the inactive lever. The R278995/CRA0450 decreased stress-induced responding on the active lever [Figure 4A;  $F(2,22) = 3.908, p < .035$ ] and did not affect responding on the inactive lever.

### Experiment 4: Effect of Astressin-2B on Stress-Induced Reinstatement of Nicotine-Seeking

The mean ( $\pm$  SEM) number of infusions of .03 mg/kg of nicotine, responses on the active lever, and responses on the inactive lever during the last day of nicotine self-administration were  $14.94 \pm 1.72$ ,  $75.06 \pm 8.49$ , and  $12.56 \pm 2.80$ , respectively. The role of the CRF<sub>2</sub> receptor in stress-induced reinstatement of nicotine-seeking was investigated in two separate groups of 8 rats. Substituting saline for nicotine caused a rapid decline in the number of responses on the active lever in the first group



**Figure 2.** Effect of the corticotropin-releasing factor (CRF)<sub>2</sub> receptor antagonist astressin-2B (saline,  $n = 8$ ; nicotine,  $n = 8$ ) on the elevations in brain reward thresholds associated with mecamylamine-precipitated nicotine withdrawal (A). Effect of astressin-2B on the response latencies of rats chronically treated with saline ( $n = 8$ ) or nicotine ( $n = 8$ ) and acutely treated with mecamylamine (B). Brain reward thresholds and response latencies are expressed as a percentage of the pre-test day values. Asterisks ( $*p < .05$ ,  $**p < .01$ ) indicate elevations in brain reward thresholds compared with those of the corresponding saline-treated control group. Data are expressed as means  $\pm$  SEM.

[ $F(13,91) = 5.152, p < .0001$ ] and the second group [ $F(6,42) = 3.553, p < .006$ ]. The extinction criterion ( $< 2$  infusions) was met after 14 days in the first group and after 7 days in the second group. During the extinction period the number of responses on the inactive lever decreased in the first group [ $F(13,91) = 3.836, p < .0001$ ] and the second group [ $F(6,42) = 3.868, p < .004$ ]. For further data analyses the first and second groups were combined. The administration of footshocks increased the number of responses on the active lever [last day of extinction vs. footshocks vehicle condition;  $t(15) = 7.70, p < .0001$ ] and did not affect the number of responses on the inactive lever. Astressin-2B did not affect footshock-induced responding on the active lever or the inactive lever (Figure 4B). This suggests that blockade of the CRF<sub>2</sub> receptor does not affect stress-induced reinstatement of nicotine-seeking.

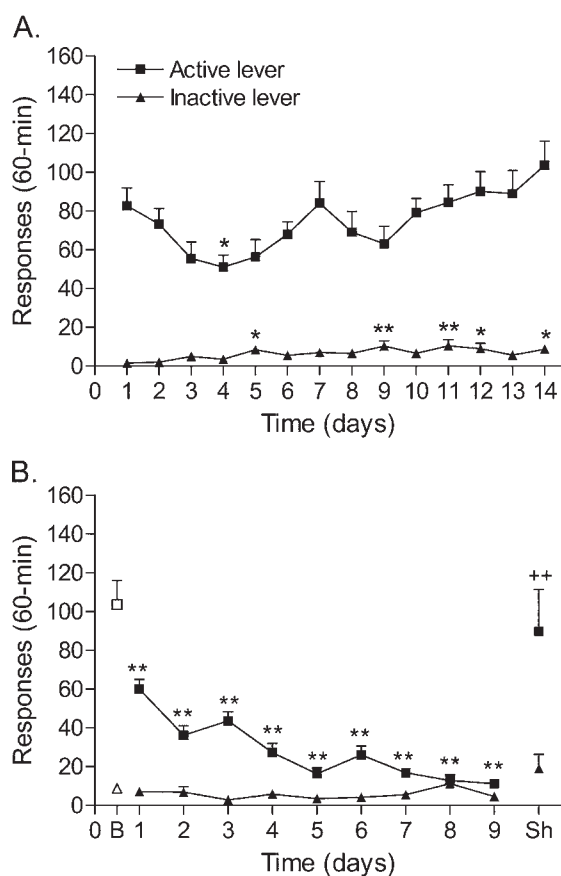
### Experiment 5: Effects of R278995/CRA0450 and Astressin-2B on Responding for Food Pellets

Table 1 shows the effects of R278995/CRA0450 and astressin-2B on responding for food pellets under an FR5 TO20-s schedule of reinforcement. The CRA0450/R278995 or astressin-

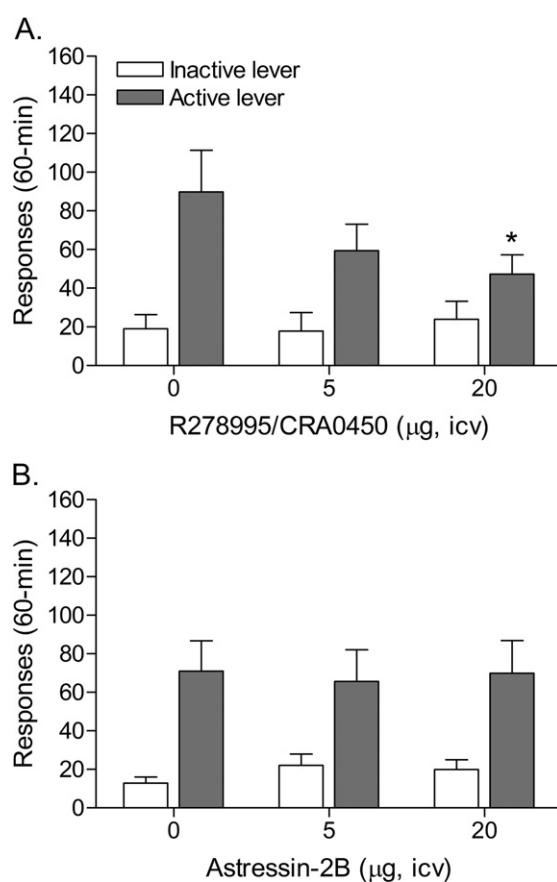
sin-2B did not affect responding on the active or the inactive lever.

## Discussion

The CRF<sub>1</sub> receptor antagonist R278995/CRA0450 prevented the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. In contrast, the CRF<sub>2</sub> receptor antagonist astressin-2B did not affect the elevations in brain reward thresholds associated with precipitated nicotine withdrawal. Furthermore, the CRF<sub>1</sub> receptor antagonist R278995/CRA0450 but not the CRF<sub>2</sub> receptor antagonist astressin-2B prevented stress-induced reinstatement of extinguished nicotine-seeking. Neither R278995/CRA0450 nor astressin-2B affected operant responding for food pellets. This indicates that neither compound induced motor impairments or sedative effects. The present findings extend and corroborate previous findings by demonstrating that the nicotine withdrawal-induced deficit in brain reward function and stress-induced reinstatement of nicotine-seeking are at least partly mediated by the activation of central CRF<sub>1</sub> receptors.



**Figure 3.** Responding on the active and inactive lever during nicotine self-administration (A) ( $n = 12$ ), extinction training, and relapse (B) ( $n = 12$ ). In (A) asterisks ( $*p < .05$ ,  $**p < .01$ ) indicate a decrease in responding on the active lever or an increase in responding on the inactive lever compared with the first day of nicotine self-administration. In (B) asterisks ( $**p < .01$ ) indicate a decrease in responding on the active lever compared with baseline responding (last day of nicotine self-administration). Plus signs ( $+p < .01$ ) indicate a footshock-induced increase in responding on the active lever compared with day 9 of extinction training. Data are expressed as means  $\pm$  SEM. B, baseline; Sh, footshocks.



**Figure 4.** Effects of the corticotropin-releasing factor (CRF)<sub>1</sub> receptor antagonist R278995/CRA0450 (A) ( $n = 12$ ) and the CRF<sub>2</sub> receptor antagonist astressin-2B (B) ( $n = 16$ ) on footshock-induced reinstatement of nicotine-seeking behavior. Asterisks ( $*p < .05$ ) indicate a decrease in responding on the active lever compared with rats that were pretreated with vehicle. Data are expressed as means  $\pm$  SEM.

Extensive evidence suggests that a hyperactivity of brain CRF systems plays a role in negative emotional states (38). Substantial evidence also points toward a role for brain stress systems in drug addictions. Alcohol, nicotine, and cannabinoid withdrawal increases extracellular CRF levels in the central nucleus of the amygdala (24,39,40). The withdrawal-induced increase in CRF levels has been suggested to mediate increased anxiety-like behavior and drug intake in dependent animals (41,42). The nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonist  $\alpha$ -helical CRF<sub>(9-41)</sub> has been shown to decrease alcohol withdrawal-induced anxiety-like behavior in rats (43). In addition, specific CRF<sub>1</sub> receptor antagonists decrease alcohol intake in alcohol-dependent animals (25). In a previous study we reported that the nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonist D-Phe CRF<sub>(12-41)</sub> prevents the elevations in brain reward thresholds associated with nicotine withdrawal (21). The results presented here indicate that CRF mediates the nicotine withdrawal-induced deficit in brain reward function at least partly by activating central CRF<sub>1</sub> receptors. These findings suggest that antagonism of CRF<sub>1</sub> receptors could be an efficacious treatment for the anhedonic-state associated with smoking cessation. Although a great number of studies have investigated the role of CRF<sub>2</sub> receptors in anxiety-like behavior (42,44–46), very few studies have reported on the role of CRF<sub>2</sub> receptors in drug withdrawal. Evidence indicates that the activation of CRF<sub>2</sub> receptors diminishes alcohol withdrawal-induced

**Table 1.** Effects of R278995/CRA0450 and Astressin-2B on Food Responding

Dose ( $\mu\text{g}$ , ICV)	Active Lever	Inactive Lever
R278995/CRA0450		
0	249.3 $\pm$ 13.9	2.7 $\pm$ 1.7
5	260.5 $\pm$ 17.7	.5 $\pm$ .2
20	256.0 $\pm$ 30.1	1.9 $\pm$ .9
Astressin-2B		
0	244.2 $\pm$ 7.3	.8 $\pm$ .4
5	241.6 $\pm$ 10.8	1.4 $\pm$ .8
20	246.1 $\pm$ 4.5	1.7 $\pm$ .8

Data are expressed as means  $\pm$  SEM.

anxiety-like behavior (26). In contrast, somatic morphine withdrawal signs are attenuated in CRF<sub>2</sub> receptor-deficient mice, thus suggesting that CRF<sub>2</sub> receptor activation contributes to withdrawal symptomatology (47). In a previous study we reported that blockade of CRF<sub>1</sub>/CRF<sub>2</sub> receptors prevents the negative affective state associated with nicotine withdrawal, and in the present study we demonstrated that a CRF<sub>1</sub> receptor antagonist but not a CRF<sub>2</sub> receptor antagonist prevents the negative affective state associated with nicotine withdrawal (21). Therefore, these studies suggest that CRF<sub>2</sub> receptors do not play a role in the deficit in brain reward function associated with nicotine withdrawal. The ICV administration of CRF elevates brain reward thresholds and induces conditioned place aversion, and these effects can be prevented by pretreatment with nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonists (48,49). Although there is strong evidence for a role of CRF<sub>1</sub> receptors in negative affective states (41), a recent study reported that blockade of CRF<sub>2</sub> receptors but not CRF<sub>1</sub> receptors prevents ICV CRF-induced conditioned place aversion (50). This would suggest that drug withdrawal-induced negative affective states are mediated via the activation of CRF<sub>1</sub> receptors and exogenous CRF might mediate negative affective states via the activation of CRF<sub>2</sub> receptors. A possible explanation for this discrepancy could be that exogenous and endogenous CRF might stimulate different CRF receptor populations. Exogenous CRF might induce activation of CRF receptors that are located in close proximity to the lateral ventricles (e.g., CRF<sub>2</sub> receptors in lateral septum). In contrast, drug withdrawal might lead to the activation of CRF<sub>1</sub> receptors in very specific brain sites such as the central nucleus of the amygdala (24,39,40).

Stressors play an important role in relapse to drug abuse in humans. Footshocks have been shown to induce the reinstatement of extinguished drug-seeking behavior in rats (14,20,51). Footshocks activate brain CRF systems, as indicated by an increased release of CRF in the ventral tegmental area and an increased expression of c-Fos protein in CRF-immunoreactive neurons (52,53). In a previous study, we reported that the CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonist D-Phe CRF<sub>(12-41)</sub> prevents stress-induced reinstatement of nicotine-seeking (22). This follow-up study suggests that footshocks mediate stress-induced reinstatement of nicotine-seeking via the activation of CRF<sub>1</sub> and not CRF<sub>2</sub> receptors. The outcome of this study suggests that small-molecule CRF<sub>1</sub> receptors antagonists might decrease the risk for stress-induced relapse to smoking. The outcome of this study is in line with previous studies that investigated the role of CRF<sub>1</sub> receptors in stress-induced reinstatement of drug-seeking. Blockade of CRF<sub>1</sub> receptors has been shown to attenuate stress-induced reinstatement of heroin-, cocaine-, and alcohol-seeking (18,19). Furthermore, blockade of CRF<sub>1</sub> receptors attenuates stress-induced reinstatement of morphine and cocaine-induced

conditioned place preference (54,55). The ICV administration of a CRF<sub>2</sub> receptor antagonist does not attenuate stress-induced reinstatement of cocaine-induced conditioned place preference (55). A recent study by Wang *et al.* (56) suggests that blockade of CRF<sub>2</sub> receptors but not CRF<sub>1</sub> receptors in the ventral tegmental area prevents footshock-induced reinstatement of cocaine-seeking. The latter study suggests that the administration of specific CRF receptor antagonists into brain sites might have a different effect on stress-induced reinstatement of drug-seeking than the ICV administration of CRF receptor antagonists. Follow-up studies are needed to investigate whether CRF<sub>2</sub> receptors in the ventral tegmental area also play a critical role in stress-induced reinstatement of nicotine-seeking. Drugs and cues associated with the self-administration of drugs of abuse can induce the reinstatement of drug-seeking (57). Evidence suggests that CRF<sub>1</sub> receptor activation might play a role in drug- and cue-induced reinstatement of drug-seeking. The nonspecific CRF<sub>1</sub>/CRF<sub>2</sub> receptor antagonist D-Phe CRF<sub>(12-41)</sub> attenuates drug-induced reinstatement of cocaine-seeking (58). Furthermore, the specific CRF<sub>1</sub> receptor antagonist CP-154,526 blocks cue- and drug-induced reinstatement of cocaine- and methamphetamine seeking (59,60). Additional studies are warranted to investigate whether CRF<sub>1</sub> receptors also play a role in cue- and drug-induced reinstatement of nicotine-seeking.

The present studies demonstrated that R278995/CRA0450 prevents stress-induced reinstatement of extinguished nicotine-seeking. It is unlikely that R278995/CRA0450 prevented stress-induced nicotine-seeking by inhibiting motor output. The dose of R278995/CRA0450 (20  $\mu\text{g}$ , ICV) that prevented stress-induced reinstatement of nicotine-seeking did not affect the response latencies in the ICSS procedure and did not decrease operant responding for food pellets. In the present studies we did not investigate the effect of R278995/CRA0450 on baseline operant responding in rats in which intravenous nicotine self-administration was extinguished. However, on the basis of the aforementioned findings, it is extremely unlikely that R278995/CRA0450 would have affected baseline responding. Furthermore, a previous study reported that doses of the CRF<sub>1</sub> receptor antagonist CP-154,526 that attenuated stress-induced reinstatement of extinguished heroin- and cocaine-seeking did not affect baseline responding in animals in which drug-seeking was extinguished (19).

Taken together, these findings indicate that blockade of CRF<sub>1</sub> receptors prevents the deficit in brain reward function associated with nicotine withdrawal and stress-induced reinstatement of extinguished nicotine-seeking. These studies also suggest that activation of CRF<sub>2</sub> receptors does not play a role in acute nicotine withdrawal and stress-induced reinstatement of nicotine-seeking. These studies have significant clinical implications for the treatment of tobacco addiction. The present findings suggest that small-molecule CRF<sub>1</sub> receptor antagonists might diminish the anhedonic-state associated with smoking cessation, prevent stress-induced reinstatement of tobacco smoking, and thereby could improve smoking cessation rates.

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