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Effects of the combination of metyrapone and oxazepam on intravenous nicotine self-administration in rats

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Abstract

Rationale—Despite increased education regarding its dangers, cigarette smoking remains a significant public health concern due to serious associated health consequences such as cancer and respiratory and cardiovascular diseases. Most smokers fail in their attempts to quit smoking, and current pharmacological interventions have relatively low levels of efficacy and are associated with significant adverse events. We have previously reported that combinations of metyrapone and oxazepam, administered at doses that were ineffective when delivered singly, resulted in dose-related decreases in cocaine self-administration in rats while not affecting food-maintained responding during the same sessions.

Objectives—The current study was designed to test the effects of the administration of a metyrapone:oxazepam combination on nicotine self-administration in rats.

Methods—Several dose combinations of metyrapone (12.5, 25 or 50 mg/kg) and oxazepam (5 or 10 mg/kg) were tested in rats trained to intravenously (IV) self-administer nicotine (0.03 mg/kg/infusion) during 1-h self-administration sessions using both fixed-ratio and progressive-ratio (PR) schedules of reinforcement.

Results—The administration of low doses of metyrapone and oxazepam in combination significantly decreased IV nicotine self-administration in rats. At the lowest doses of 12.5 mg/kg

of metyrapone and 5 mg/kg of oxazepam, the drugs alone did not decrease IV nicotine self-administration, but the combination was effective. Varenicline was also tested using the fixed-ratio schedule, and reductions in nicotine intake were similar to those seen with the moderate dose of the combination.

Conclusions—The results of this study suggest a potential utility of the combination of metyrapone and oxazepam for smoking cessation in humans.

Keywords

Nicotine; Self-administration; Metyrapone; Oxazepam; Reinforcement; Varenicline; HPA axis; Benzodiazepine; Corticotropin-releasing factor; Rat

Introduction

Despite increases in knowledge among the general population regarding the significant risks associated with cigarette smoking, nicotine dependence continues to be a major public health concern due to serious associated consequences such as cancer, respiratory and cardiovascular diseases (Doll et al. 2004; Peto et al. 2000). Most smokers fail in their attempts to quit smoking, especially without pharmacological and/or psychological help. The chances of quitting increase significantly when individuals employ pharmacological approaches such as nicotine replacement therapy (Moore et al. 2009; Ray et al. 2007) and the antidepressant bupropion (Paterson 2009; Wilkes 2008). More recently, the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist varenicline (Keating and Lyseng-Williamson 2010) has been reported to increase efficacy for smoking cessation compared to either bupropion (Cahill et al. 2009; Gonzales et al. 2006; Jorenby et al. 2006) or nicotine replacement therapy (Aubin et al. 2008; Cahill et al. 2009). However, there are reports of adverse events with varenicline (McNeil et al. 2010) including suicidal ideation and behavior (Harrison-Woolrych 2009; Moore and Furberg 2009). In 2008, the FDA issued a safety warning about the neuropsychiatric symptoms associated with the drug (i.e., changes in behavior, agitation, suicidal ideation and attempted and completed suicide). Thus, there is a continuing need for the development of safe and effective pharmacotherapies for the treatment of nicotine dependence.

Addiction to tobacco is a complex disorder, although there is general agreement that the dependence associated with tobacco smoking is, in part, mediated by the primary reinforcing properties of nicotine (Le Foll and Goldberg 2006; Rose et al. 2007). However, one of the many factors contributing to smoking relapse following smoking cessation is stress (Albertsen et al. 2006; Kassel et al. 2003; Pomerleau and Pomerleau 1991; West 2009). Over the last several years, we and a number of other researchers have explored the complex relationship between stress and the subsequent activation of the hypothalamic–pituitary–adrenal (HPA) axis in drug reward (Goeders 2002; Goeders 2007; Koob and Kreek 2007; Lowery and Thiele 2010; Majewska 2002; Silberman et al. 2009) including nicotine (Pickworth and Fant 1998). Our research has primarily focused on psychomotor stimulants, and we have investigated the effects of drugs that alter HPA axis activity on cocaine self-administration and cue reactivity associated with cocaine- and methamphetamine-seeking in rats (Goeders 2004; Goeders 2007). We initially investigated the effects of benzodiazepines on intravenous cocaine self-administration in rats. Benzodiazepines decrease plasma corticosterone (Keim and Sigg 1977), and cortisol (Meador-Woodruff and Greden 1988; Torpy et al. 1993) in rats and humans, respectively, and attenuate cocaine-induced increases in plasma corticosterone in rats (Yang et al. 1992). We have shown that the benzodiazepines chlordiazepoxide (Goeders et al. 1989) and alprazolam (Goeders et al. 1993) are effective in reducing cocaine self-administration in rats. We have also investigated the effects of

corticosterone synthesis inhibitors on cocaine self-administration. Metyrapone (Haleem et al. 1988; Haynes 1990) and ketoconazole (Engelhardt et al. 1985) block the 11 β -hydroxylation reaction in the production of cortisol/corticosterone to decrease plasma concentrations of the hormone, and we have shown that these drugs also reduce cocaine taking in rats (Goeders and Guerin 1996; Goeders et al. 1998).

The activity of benzodiazepines and corticosterone synthesis inhibitors in these animal models of drug self-administration suggests that these drugs may prove useful in treating drug-dependent humans. However, both classes of drugs have potential side effects that could limit their usefulness. For example, benzodiazepines are not usually recommended for use in drug-dependent individuals since these drugs have the potential for dependence and abuse (Chouinard 2004; Lilja et al. 2001; O'Brien 2005). Cortisol synthesis inhibitors have the potential to produce adrenal insufficiency, also potentially limiting the utility of this class of drugs.

To address these concerns, we reasoned that the incidence of side effects produced by these two classes of drugs may be mitigated by reducing the dose. Our hypothesis was that by combining drugs that affect HPA axis activity through different mechanisms and delivering these drugs at doses that have no effect when administered alone, we would minimize their potential toxic and unwanted side effects while still reducing cocaine intake. We confirmed this hypothesis in a rat model of cocaine self-administration by demonstrating that combinations of metyrapone and oxazepam, administered at doses that were ineffective when delivered singly, resulted in dose-related decreases in cocaine self-administration in rats (Goeders and Guerin 2008). These same combinations did not affect food-maintained responding during the same sessions.

Our underlying hypothesis regarding the actions of metyrapone and oxazepam is that rather than blocking distinct physiological processes associated with reward or reinforcement (e.g., dopamine) in response to a specific drug such as cocaine, this combination may, in more general terms, block the role of the activation of the arousal–stress axis in facilitating reward and associated drug use and relapse (Pecina et al. 2006). If our hypothesis is correct, then the combination of metyrapone and oxazepam should be useful for reducing the intake of other drugs that are also associated with HPA axis activity. Thus, we hypothesized that the combination of metyrapone and oxazepam would be effective in decreasing the self-administration of nicotine, another addictive drug with a different mechanism of action than cocaine or methamphetamine. In this paper, we report that the combination of metyrapone and oxazepam reduces intravenous (IV) nicotine self-administration in rats responding under fixed-ratio and progressive-ratio (PR) schedules of reinforcement.

Materials and methods

Animals

Wistar-derived male rats (250–300 g) were purchased from Harlan Laboratories (Livermore, CA). Rats were housed in groups of two and maintained in a temperature-controlled environment on a 12 h:12 h light cycle (0600 h on–1800 h off) after receipt. Before experimental testing, animals were provided free access to food and water during a 1-week habituation period and were handled daily for several days to desensitize them to handling stress. Animals were identified by cage number and by numbers applied to the proximal tip of the tail using a “Sharpie R” permanent marker. Animals were handled, housed and euthanized in accord with the current NIH guidelines regarding the use and care of laboratory animals and all applicable local, state, and federal regulations and guidelines. Each rat included in the data analysis received all treatments.

Drug treatments

In the present study, rats received one of several dose combinations of metyrapone (12.5, 25 or 50 mg/kg, Sigma-Aldrich) and oxazepam (5 or 10 mg/kg, Sigma-Aldrich) or vehicle administered intraperitoneally as a suspension containing 5% Alkamuls EL-620 (Rhodia) in 0.9% saline. Varenicline tartrate (Ontario Chemicals) at 1 mg/kg (expressed as free base) was used as a positive control and administered subcutaneously in 0.9% saline. Nicotine hydrogen tartrate (Sigma-Aldrich) was dissolved in isotonic saline at 0.3 mg/ml (expressed as free base), adjusted to pH 7.0 and diluted to the appropriate concentration to deliver 0.03 mg/kg/infusion. All test compounds were administered 30 min prior to nicotine self-administration sessions in a volume of 1 ml/kg. The doses tested in the first fixed-ratio study were: 50 mg/kg metyrapone:10 mg/kg oxazepam, 50 mg/kg metyrapone:5 mg/kg oxazepam, 25 mg/kg metyrapone:5 mg/kg oxazepam and 1 mg/kg varenicline. The combination of 25 mg/kg metyrapone:5 mg/kg oxazepam was also tested in the PR experiment. We selected these doses based on our previous data in a rat model of cocaine self-administration, in which the doses of this combination reduced drug intake without affecting food-maintained responding during the same sessions (Goeders and Guerin 2008). To ascertain whether metyrapone or oxazepam had effects on their own compared to the combination on nicotine self-administration, a second fixed-ratio study was conducted in which the doses were: 25 mg/kg metyrapone:5 mg/kg oxazepam and 12.5 mg/kg metyrapone:5 mg/kg oxazepam in combination and 12.5 and 25 mg/kg metyrapone and 5 mg/kg oxazepam tested alone.

Apparatus

Food training and nicotine self-administration took place in eight standard Coulbourn self-administration conditioning chambers. Each chamber was housed in a sound-attenuating chamber. The self-administration chambers were equipped with two levers mounted 2 cm above the floor and a cue light mounted 2 cm above the right lever (active lever) on the back wall of the chamber. For food training, a food hopper was located 2 cm to the left/right of either lever in the middle of the back wall. Intravenous infusions were delivered in a volume of 0.1 ml over a 1-s interval via a motor-driven infusion pump (Razel) housed outside of the sound-attenuating chamber.

Food training

Lever pressing was established as demonstrated previously (Hyytia et al. 1996). Initially, rats were restricted to 15 g of food daily to maintain them at approximately 85% of their free-feeding body weights. After the second day of food restriction, rats were trained to respond under a fixed-ratio 1 (FR1) schedule of food reinforcement (i.e., one food pellet was delivered following each lever press) with a 1-s time-out (TO-1 s) following each pellet delivery, and the response requirement was gradually increased to a FR1-TO-20s schedule of reinforcement. Training sessions were administered twice daily (0900 h and 1500 h), and each session lasted for 30 min. Once rats obtained steady baseline responding at a FR1-TO-20s schedule of reinforcement for food, defined as less than 20% variability across three consecutive sessions, they were returned to ad libitum feeding in preparation for surgical implantation of the jugular catheter.

Surgery

Rats were anesthetized with an isoflurane–oxygen mixture (1–3% isoflurane), and a chronic silastic jugular catheter was inserted into the external jugular vein and passed subcutaneously to a polyethylene assembly mounted on the animal's back (Koob and Goeders 1989). The catheter assembly consisted of a 13-cm length of silastic tubing (inside diameter 0.31 mm; outside diameter 0.64 mm), attached to a guide cannula bent at a right angle. The cannula was embedded in a dental cement base and anchored with a 2 × 2 cm

square of durable mesh. The catheter was passed subcutaneously from the back to the jugular vein where it was inserted and secured with a nonabsorbable silk suture. Upon successful completion of surgery, rats were given 5 days to recover before baseline self-administration sessions started. During the recovery period, rats remained on ad libitum food access and had their catheter lines flushed daily with saline containing 30 U/ml of heparin and 66 mg/ml of Timentin to prevent blood coagulation and infection.

Nicotine self-administration

Following successful recovery from surgery, rats ($N=8$) were again food deprived to 85% of their free-feeding body weights and were trained to self-administer nicotine (0.03 mg/kg/infusion, IV) during 1-h baseline sessions conducted 5 days per week under a FR1-TO-20s schedule of reinforcement until stable responding was achieved. Stable responding was defined as less than 20% variability across two consecutive sessions, which was achieved after 11 baseline sessions. The various dose combinations of metyrapone and oxazepam or vehicle were then tested using a within-subjects Latin square design (LSD). After the testing of a dose, rats were allowed to reestablish stable baseline responding before the next dose was tested. Following the within-subjects dose testing with metyrapone and oxazepam, rats were run under baseline conditions for a minimum of 5 days until stable responding was achieved, after which the positive control varenicline was tested. Upon completion of varenicline testing, rats were again run under baseline conditions for a minimum of 5 days until stable responding was achieved.

The same rats were then tested using a PR schedule of reinforcement, with each nicotine infusion resulting in a progressive increase in the number of lever presses required to obtain the subsequent infusion. The progression of lever presses was as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, etc., derived from the formula $((5 - \epsilon 0.2n) - 5)$ rounded to the nearest integer where n is the position in the sequence of ratios. For PR testing, half of the rats were tested with metyrapone and oxazepam and the other half tested with vehicle on PR day 1. Following PR day 1, rats were run under baseline conditions (FR1-TO-20) at 0.03 mg/kg/infusion until stable responding was again observed. A second PR session was then conducted with rats that received drug treatment in the first PR session receiving vehicle and rats that received vehicle in the first PR session receiving metyrapone and oxazepam. PR sessions were programmed for duration of 6 h, and if rats did not produce a “reinforced response” within 1 h, sessions were terminated and breakpoints were determined based on the last reward achieved under this condition.

A separate group of rats ($N=6$) was trained to self-administer nicotine (0.03 mg/kg/infusion, IV) under the FR1-TO-20s schedule of reinforcement as described above. When stable responding was achieved, the rats were tested with metyrapone (12.5 or 25 mg/kg) and oxazepam (5 mg/kg) ip alone and then in combination (12.5 mg/kg metyrapone:5 mg/kg oxazepam; 25mg/kgmetyrapone:5mg/kg oxazepam). Rats were allowed to reestablish stable baseline responding before the next dose was tested. At the end of the second FR experiment, four rats underwent extinction. Here, during each extinction session (1 h per day), the motors of the pumps were turned on when an active lever press was made, but no drug was delivered and the cue light did not turn on.

Catheters were flushed with saline before each test session to ensure catheter patency, and the catheters were flushed again after each test session with saline containing 30 U/ml of heparin and 66 mg/ml of Timentin to prevent blood coagulation and infection in the catheters. If catheter patency was in question (e.g., demonstrated by an unexpected shift in response rates or an inability to draw blood from the catheter), 0.1 ml of a short-acting anesthetic (i.e., Brevital, 10 mg/ml) was infused. Animals with patent catheters exhibited a loss of muscle tone within 3 s. Rats with catheters no longer patent according to the Brevital

test were removed from the experiment. Based upon this test, one rat was removed during the first study due to catheter failure; the data shown represent the responses of eight rats that completed testing with all test agents.

Data compilation, processing and analysis

Data were collected online simultaneously from multiple self-administration chambers. Data from the nicotine self-administration experiments were reported as the percent of baseline responding or mean cumulative number of nicotine-reinforced responses. Results from the PR study were reported as the mean number of reinforced responses and the breakpoint. In general, tests for homogeneity of variance were first performed on the data. If the scores did not violate the assumption of homogeneity of variance, appropriate analyses of variance (ANOVA) were performed. Test data were analyzed using the StatView statistical package on a PC-compatible computer. For the analysis of all dose–response curves, a repeated measures ANOVA was conducted. Follow-up analyses (i.e., Newman–Keuls post hoc testing) were conducted where appropriate.

Results

Evidence that nicotine maintained self-administration behavior

Under control conditions (vehicle treatment), rats received an average of 15.0 ± 1.5 infusions of nicotine over the 1-h test session under the FR1-TO-20s schedule of reinforcement. Nicotine also reliably sustained PR responding with a mean breakpoint of 12.6 ± 1.6 (as evident by stable PR responding during both PR sessions (in vehicle-treated rats) in the crossover drug administration design), a value similar to that published for heroin in nondependent rats (Hubner and Koob 1990). Finally, all four animals that were tested at the end of the second FR experiment showed suppressed responding on the active lever when the nicotine infusions were omitted, reaching the criteria of extinction (less than 30% of the active lever presses made during baseline sessions, on two consecutive extinction sessions) within 13–16 days.

Effects of the combination of metyrapone and oxazepam on IV nicotine self-administration: fixed-ratio schedule

The effects of pretreatment with the combination of metyrapone and oxazepam on nicotine self-administration using a fixed-ratio schedule are depicted in Fig. 1. Treatment with the combination of metyrapone:oxazepam reduced nicotine self-administration in a dose-related fashion, with dose ratios of 25:5 mg/kg, 50:5 mg/kg and 50:10 mg/kg reducing nicotine infusions to 7.1 ± 1.6 , 5.3 ± 1.3 and 3.1 ± 1.0 , respectively.

An ANOVA of treatment groups revealed that the effects of metyrapone:oxazepam on nicotine intake relative to vehicle were significant [$F(3,21)=16.970$, $p<0.0001$]. Post hoc analyses of the combinations of metyrapone and oxazepam revealed that the results for all dose combinations were significantly different from those obtained with vehicle (metyrapone:oxazepam, 25:5 mg/kg, $p=0.0091$; 50:5 mg/kg, $p=0.008$; 50:10 mg/kg, $p<0.0001$). Analysis of order effects using the average value of all rats within each treatment day found no significant findings.

Effects of metyrapone and oxazepam alone and in combination on IV nicotine self-administration: fixed-ratio schedule

This experiment examined whether oxazepam, metyrapone or the combination of the two drugs would reduce nicotine intake on an FR1 schedule of self-administration. All rats were first tested with 5 mg/kg oxazepam alone and 25 mg/kg metyrapone alone, and the effects of pretreatment with these doses of metyrapone and oxazepam alone and in combination on

nicotine self-administration are depicted in Fig. 2a. Under control conditions (vehicle treatment), rats received an average of 18.6 ± 3.0 infusions of nicotine over the 1-h test session under the FR1-TO-20s schedule of reinforcement. An ANOVA of the treatment conditions revealed that treatment with both metyrapone alone and the metyrapone:oxazepam combination significantly reduced ($F(3,4)=10.66$; $P<0.01$) (Newman-Keuls; $P<0.05$) nicotine self-administration. Baseline responding was then reestablished (22.2 ± 2.8 infusions/session), and the experiment was repeated, testing doses of 12.5 mg/kg metyrapone and 5 mg/kg oxazepam, alone and in combination (Fig. 2b). This time, only the metyrapone:oxazepam combination significantly reduced nicotine intake ($F(3,4)=7.4$; $P<0.01$) (Newman-Keuls; $P<0.05$). The effects of these drugs alone and in combination on inactive lever responding were also determined, and no significant effects on responding were found (Table 1).

Effects of varenicline on IV nicotine self-administration

The effects of 1.0 mg/kg varenicline pretreatment on nicotine self-administration are depicted in Fig. 1. Varenicline treatment was found to reduce nicotine infusions from 15.4 ± 1.0 to 7.7 ± 1.2 . An ANOVA of these data revealed that this reduction in the number of nicotine infusions during the 1-h self-administration session was statistically significant [$F(1,7)=47.042$, $p<0.0003$]. A comparison of the varenicline results with those seen with metyrapone: oxazepam showed that the 25:5 metyrapone:oxazepam dose combination was comparable to varenicline at reducing nicotine self-administration.

Effects of the combination of metyrapone and oxazepam on IV nicotine self-administration: PR schedule

The 25:5 mg/kg metyrapone:oxazepam combination was also tested under the PR schedule. As shown in Fig. 3a, pretreatment with this combination of metyrapone and oxazepam reduced the number of nicotine infusions from 6.1 ± 0.5 to 2.8 ± 0.6 . The breakpoint was also reduced from 12.6 ± 1.6 with vehicle to 3.9 ± 0.9 with metyrapone:oxazepam (Fig. 3b). An ANOVA of the PR data revealed that pretreatment with 25:5 mg/kg metyrapone:oxazepam resulted in a significant decrease in the total number of nicotine infusions [$F(1,7)=15.997$, $p<0.0055$] and the breakpoint [$F(1,7)=19.533$, $p<0.0035$]. Varenicline was not tested under the PR schedule.

Discussion

The results of this investigation demonstrate that combinations of low doses of metyrapone and oxazepam decrease IV nicotine self-administration in rats in a dose-related manner. Although we only investigated a single dose of nicotine (0.03 mg/kg/infusion), we have previously shown that this drug combination is effective across several doses of cocaine (Goeders and Guerin 2008). Significant reductions in nicotine self-administration were even seen with the lowest dose combination tested, suggesting that nicotine self-administration may be even more sensitive to the effects of the combination than cocaine self-administration (Goeders and Guerin 2008). Although the testing of metyrapone and oxazepam as single agents was only conducted in the second fixed-ratio experiment, all doses of metyrapone and oxazepam chosen for testing as a combination in this study have no effect on cocaine self-administration when delivered as single agents. Furthermore, these same combinations have no effect on food self-administration during the same session (Goeders and Guerin 2008), suggesting that these effects were not the result of nonspecific effects on the ability of the rats to respond. The 25:5 mg/kg combination of metyrapone and oxazepam also reduced nicotine self-administration maintained under a PR schedule of reinforcement, suggesting that the combination of metyrapone and oxazepam reduces the motivation to seek and take nicotine. Finally, the effects of the combination of metyrapone

and oxazepam were comparable to those of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist varenicline in this specific model of intravenous nicotine self-administration (George et al. 2011; Keating and Lyseng-Williamson 2010; O'Connor et al. 2010). Since varenicline is effective in reducing cigarette smoking in humans (Keating and Lyseng-Williamson 2010) and appears to be more beneficial than either bupropion (Cahill et al. 2009; Gonzales et al. 2006; Jorenby et al. 2006) or nicotine replacement therapy (Aubin et al. 2008; Cahill et al. 2009), these data suggest that the combination of metyrapone and oxazepam may also be effective in reducing smoking (O'Dell and Khroyan 2009).

The mechanisms mediating the effects of the combination of metyrapone and oxazepam on nicotine self-administration are unclear at this time. Metyrapone blocks the 11β -hydroxylation reaction in the synthesis of corticosterone to decrease plasma concentrations of the hormone (Haleem et al. 1988; Haynes 1990). Benzodiazepines can also reduce the elevated cortisol secretion often seen in some psychiatric disorders (Keim and Sigg 1977; Meador-Woodruff and Greden 1988; Torpy et al. 1993) and inhibit the cortisol response to adrenocorticotrophic hormone (Grottoli et al. 2002). Surprisingly, however, the combination of these low doses of metyrapone and oxazepam do not alter plasma corticosterone in rats (Goeders and Guerin 2008), suggesting that the behavioral effects of this combination are mediated by mechanisms not necessarily reflected through this hormone. One hypothesis is that these results may be mediated through effects on brain corticotropin-releasing factor (CRF). Corticosterone synthesis inhibitors (i.e., ketoconazole and metyrapone) and benzodiazepines have all been shown to alter (increase) CRF in various brain regions (Smagin and Goeders 2004; Van Vugt et al. 1997; Wilson et al. 1996).

There is a general consensus that a relationship between cigarette smoking and stress exists. Smoking increases under stressful conditions (Pomerleau and Pomerleau 1991) and is maintained, in part, due to the stress and negative affect that arise during the withdrawal that occurs between cigarettes (Heishman 1999; Kassel et al. 2003). In humans, cigarette smoking increases plasma concentrations of cortisol and adrenocorticotrophic hormone (Mendelson et al. 2008; Mendelson et al. 2005), and the increases in these hormones correlate positively with reductions in craving. In chronic smokers, smoking produces a somewhat attenuated HPA axis response to psychological stressors although cortisol is still increased in response to an injection of CRF (Rohleder and Kirschbaum 2006). These data suggest a complex relationship between cigarette smoking and HPA axis activity in humans. In rats, nicotine withdrawal augments the reactivity to light-enhanced startle responding (Jonkman et al. 2008), suggesting that nicotine withdrawal enhances the aversive effects of stress. Chronic nicotine self-administration results in tolerance to the effects of nicotine on plasma corticosterone and adrenocorticotrophic hormone in rats (Chen et al. 2008), as does chronic smoking in humans (Rohleder and Kirschbaum 2006), but the HPA responses to mild electric footshock are enhanced in rats (Chen et al. 2008). Chronic exposure to nicotine also results in conditioned increases in plasma corticosterone in response to cues paired with nicotine delivery in rats (Caggiula et al. 1998). Thus, the HPA axis may become hyporesponsive to nicotine during chronic smoking (i.e., nicotine self-administration), but HPA axis activity becomes exaggerated during withdrawal, and exposure to smoking- (nicotine-) related cues can also enhance HPA axis activity. These responses can be alleviated by another cigarette (or dose of nicotine), further highlighting the complex relationship between cigarette smoking and adrenocorticosteroids. Accumulating evidence suggests that these HPA axis responses to smoking may be related to CRF either directly or indirectly. In rats, extrahypothalamic CRF appears to be associated with the anxiety-like and anhedonia-like responses to nicotine withdrawal as well as the increase in nicotine self-administration observed following periods of abstinence (George et al. 2007; Marcinkiewicz et al. 2009). Further research will be necessary to demonstrate whether or not the

combination of metyrapone and oxazepam reduces nicotine self-administration through a CRF-related mechanism.

In summary, the administration of a combination of metyrapone and oxazepam decreases IV nicotine self-administration in rats responding under both fixed-ratio and PR schedules of reinforcement. The lowest dose of the combination tested more reliably and effectively reduced drug intake, relative to metyrapone or oxazepam alone, suggesting that the combination of metyrapone and oxazepam may be useful for smoking cessation in humans. Although the exact mechanisms mediating this effect remain to be determined, the HPA axis and extrahypothalamic CRF are possible candidates.

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References

- Albertsen K, Borg V, Oldenburg B. A systematic review of the impact of work environment on smoking cessation, relapse and amount smoked. *Prev Med.* 2006; 43:291–305. [PubMed: 16787657]
- Aubin HJ, Bobak A, Britton JR, Oncken C, Billing CB Jr, Gong J, Williams KE, Reeves KR. Varenicline versus transdermal nicotine patch for smoking cessation: results from a randomised open-label trial. *Thorax.* 2008; 63:717–724. [PubMed: 18263663]
- Caggiula AR, Donny EC, Epstein LH, Sved AF, Knopf S, Rose C, McAllister CG, Antelman SM, Perkins KA. The role of corticosteroids in nicotine's physiological and behavioral effects. *Psychoneuroendocrinology.* 1998; 23:143–159. [PubMed: 9621395]
- Cahill K, Stead L, Lancaster T. A preliminary benefit–risk assessment of varenicline in smoking cessation. *Drug Saf.* 2009; 32:119–135. [PubMed: 19236119]
- Chen H, Fu Y, Sharp BM. Chronic nicotine self-administration augments hypothalamic–pituitary–adrenal responses to mild acute stress. *Neuropsychopharmacology.* 2008; 33:721–730. [PubMed: 17551542]
- Chouinard G. Issues in the clinical use of benzodiazepines: potency, withdrawal, and rebound. *J Clin Psychiatry.* 2004; 65(Suppl 5):7–12. [PubMed: 15078112]
- Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ.* 2004; 328:1519. [PubMed: 15213107]
- Engelhardt D, Dorr G, Jaspers C, Knorr D. Ketoconazole blocks cortisol secretion in man by inhibition of adrenal 11 beta-hydroxylase. *Klin Wochenschr.* 1985; 63:607–612. [PubMed: 2993735]
- George O, Ghosland S, Azar MR, Cottone P, Zorrilla EP, Parsons LH, O'Dell LE, Richardson HN, Koob GF. CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. *Proc Natl Acad Sci USA.* 2007; 104:17198–17203. [PubMed: 17921249]
- George O, Lloyd A, Carroll FI, Damaj MI, Koob GF. Varenicline blocks nicotine intake in rats with extended access to nicotine self-administration. *Psychopharmacology (Berl).* 2011; 213:715–722. [PubMed: 20924754]
- Goeders NE. Stress and cocaine addiction. *J Pharmacol Exp Ther.* 2002; 301:785–789. [PubMed: 12023504]

- Goeders NE. Stress, motivation, and drug addiction. *Current directions in psychological science*. 2004; 13:33–35. <http://www.jstor.org/stable/20182902>.
- Goeders, NE. Hypothalamic–pituitary–adrenocortical axis and addiction. In: al'Absi, M., editor. *Stress and addiction*. London: Elsevier Neuroscience; 2007. p. 21–40.
- Goeders NE, Guerin GF. Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats. *Brain Res*. 1996; 722:145–152. [PubMed: 8813360]
- Goeders NE, Guerin GF. Effects of the combination of metyrapone and oxazepam on cocaine and food self-administration in rats. *Pharmacol Biochem Behav*. 2008; 91:181–189. [PubMed: 18692521]
- Goeders NE, McNulty MA, Mirkis S, McAllister KH. Chlordiazepoxide alters intravenous cocaine self-administration in rats. *Pharmacol Biochem Behav*. 1989; 33:859–866. [PubMed: 2559416]
- Goeders NE, McNulty MA, Guerin GF. Effects of alprazolam on intravenous cocaine self-administration in rats. *Pharmacol Biochem Behav*. 1993; 44:471–474. [PubMed: 8446681]
- Goeders NE, Peltier RL, Guerin GF. Ketoconazole reduces low dose cocaine self-administration in rats. *Drug Alcohol Depend*. 1998; 53:67–77. [PubMed: 10933341]
- Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, Watsky EJ, Gong J, Williams KE, Reeves KR. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *JAMA*. 2006; 296:47–55. [PubMed: 16820546]
- Grottoli S, Maccagno B, Ramunni J, Di Vito L, Giordano R, Gianotti L, DeStefanis S, Camanni F, Ghigo E, Arvat E. Alprazolam, a benzodiazepine, does not modify the ACTH and cortisol response to hCRH and AVP, but blunts the cortisol response to ACTH in humans. *J Endocrinol Invest*. 2002; 25:420–425. [PubMed: 12035937]
- Haleem DJ, Kennett G, Curzon G. Adaptation of female rats to stress: shift to male pattern by inhibition of corticosterone synthesis. *Brain Res*. 1988; 458:339–347. [PubMed: 2463050]
- Harrison-Woolrych M. Varenicline and suicide. Safety data from New Zealand *BMJ*. 2009; 339:b5654.
- Haynes, RC, Jr. Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones. In: Gilman, AG.; Rall, TW.; Nies, AS.; Taylor, P., editors. *The pharmacological basis of therapeutics*. New York: Pergamon; 1990. p. 1431–1462.
- Heishman SJ. Behavioral and cognitive effects of smoking: relationship to nicotine addiction. *Nicotine Tob Res*. 1999; 1(Suppl 2):S143–S147. discussion S165–146. [PubMed: 11768172]
- Hubner CB, Koob GF. The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. *Brain Res*. 1990; 508:20–29. [PubMed: 2337788]
- Hyytia P, Schulteis G, Koob GF. Intravenous heroin and ethanol self-administration by alcohol-preferring AA and alcohol-avoiding ANA rats. *Psychopharmacology (Berl)*. 1996; 125:248–254. [PubMed: 8815960]
- Jonkman S, Risbrough VB, Geyer MA, Markou A. Spontaneous nicotine withdrawal potentiates the effects of stress in rats. *Neuropsychopharmacology*. 2008; 33:2131–2138. [PubMed: 18033237]
- Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR. Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs. placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA*. 2006; 296:56–63. [PubMed: 16820547]
- Kassel JD, Stroud LR, Paronis CA. Smoking, stress, and negative affect: correlation, causation, and context across stages of smoking. *Psychol Bull*. 2003; 129:270–304. [PubMed: 12696841]
- Keating GM, Lyseng-Williamson KA. Varenicline: a pharmacoeconomic review of its use as an aid to smoking cessation. *Pharmacoeconomics*. 2010; 28:231–254. [PubMed: 20108995]
- Keim KL, Sigg EB. Plasma corticosterone and brain catecholamines in stress: effect of psychotropic drugs. *Pharmacol Biochem Behav*. 1977; 6:79–85. [PubMed: 191852]
- Koob, GF.; Goeders, NE. *Neuroanatomical substrates of drug self-administration*. London: Oxford University Press; 1989.
- Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry*. 2007; 164:1149–1159. [PubMed: 17671276]

- Le Foll B, Goldberg SR. Nicotine as a typical drug of abuse in experimental animals and humans. *Psychopharmacology (Berl)*. 2006; 184:367–381. [PubMed: 16205918]
- Lilja J, Larsson S, Skinhoj KT, Hamilton D. Evaluation of programs for the treatment of benzodiazepine dependency. *Subst Use Misuse*. 2001; 36:1213–1231. [PubMed: 11592470]
- Lowery EG, Thiele TE. Pre-clinical evidence that corticotropin-releasing factor (CRF) receptor antagonists are promising targets for pharmacological treatment of alcoholism. *CNS Neurol Disord Drug Targets*. 2010; 9:77–86. [PubMed: 20201818]
- Majewska MD. HPA axis and stimulant dependence: an enigmatic relationship. *Psychoneuroendocrinology*. 2002; 27:5–12. [PubMed: 11750767]
- Marcinkiewicz CA, Prado MM, Isaac SK, Marshall A, Rylkova D, Bruijnzeel AW. Corticotropin-releasing factor within the central nucleus of the amygdala and the nucleus accumbens shell mediates the negative affective state of nicotine withdrawal in rats. *Neuropsychopharmacology*. 2009; 34:1743–1752. [PubMed: 19145226]
- McNeil JJ, Piccenna L, Ioannides-Demos LL. Smoking cessation—recent advances. *Cardiovasc Drugs Ther*. 2010; 24:359–367. [PubMed: 20602163]
- Meador-Woodruff JH, Greden JF. Effects of psychotropic medications on hypothalamic–pituitary–adrenal regulation. *Endocrinol Metab Clin North Am*. 1988; 17:225–234. [PubMed: 2897911]
- Mendelson JH, Sholar MB, Goletiani N, Siegel AJ, Mello NK. Effects of low- and high-nicotine cigarette smoking on mood states and the HPA axis in men. *Neuropsychopharmacology*. 2005; 30:1751–1763. [PubMed: 15870834]
- Mendelson JH, Goletiani N, Sholar MB, Siegel AJ, Mello NK. Effects of smoking successive low- and high-nicotine cigarettes on hypothalamic–pituitary–adrenal axis hormones and mood in men. *Neuropsychopharmacology*. 2008; 33:749–760. [PubMed: 17507912]
- Moore TJ, Furberg CD. Varenicline and suicide. Risk of psychiatric side effects with varenicline. *BMJ*. 2009; 339:b4964. [PubMed: 19952042]
- Moore D, Aveyard P, Connock M, Wang D, Fry-Smith A, Barton P. Effectiveness and safety of nicotine replacement therapy assisted reduction to stop smoking: systematic review and metaanalysis. *BMJ*. 2009; 338:b1024. [PubMed: 19342408]
- O'Brien CP. Benzodiazepine use, abuse, and dependence. *J Clin Psychiatry*. 2005; 66(Suppl 2):28–33.
- O'Connor EC, Parker D, Rollema H, Mead AN. The alpha4-beta2 nicotinic acetylcholine-receptor partial agonist varenicline inhibits both nicotine self-administration following repeated dosing and reinstatement of nicotine seeking in rats. *Psychopharmacology (Berl)*. 2010; 208:365–376. [PubMed: 19967529]
- O'Dell LE, Khroyan TV. Rodent models of nicotine reward: what do they tell us about tobacco abuse in humans? *Pharmacol Biochem Behav*. 2009; 91:481–488. [PubMed: 19121334]
- Paterson NE. Behavioural and pharmacological mechanisms of bupropion's anti-smoking effects: recent preclinical and clinical insights. *Eur J Pharmacol*. 2009; 603:1–11. [PubMed: 19101536]
- Pecina S, Schulkin J, Berridge KC. Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical positive incentive effects in stress? *BMC Biol*. 2006; 4:8. [PubMed: 16613600]
- Peto R, Darby S, Deo H, Silcocks P, Whitley E, Doll R. Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *BMJ*. 2000; 321:323–329. [PubMed: 10926586]
- Pickworth WB, Fant RV. Endocrine effects of nicotine administration, tobacco and other drug withdrawal in humans. *Psychoneuroendocrinology*. 1998; 23:131–141. [PubMed: 9621394]
- Pomerleau OF, Pomerleau CS. Research on stress and smoking: progress and problems. *Br J Addict*. 1991; 86:599–603. [PubMed: 1859926]
- Ray R, Schnoll RA, Lerman C. Pharmacogenetics and smoking cessation with nicotine replacement therapy. *CNS Drugs*. 2007; 21:525–533. [PubMed: 17579497]
- Rohleder N, Kirschbaum C. The hypothalamic–pituitary–adrenal (HPA) axis in habitual smokers. *Int J Psychophysiol*. 2006; 59:236–243. [PubMed: 16325948]
- Rose JE, Behm FM, Salley AN, Bates JE, Coleman RE, Hawk TC, Turkington TG. Regional brain activity correlates of nicotine dependence. *Neuropsychopharmacology*. 2007; 32:2441–2452. [PubMed: 17356570]

- Silberman Y, Bajo M, Chappell AM, Christian DT, Cruz M, Diaz MR, Kash T, Lack AK, Messing RO, Siggins GR, Winder D, Roberto M, McCool BA, Weiner JL. Neurobiological mechanisms contributing to alcohol–stress–anxiety interactions. *Alcohol*. 2009; 43:509–519. [PubMed: 19913194]
- Smagin GN, Goeders NE. Effects of acute and chronic ketoconazole administration on hypothalamo–pituitary–adrenal axis activity and brain corticotropin-releasing hormone. *Psychoneuroendocrinology*. 2004; 29:1223–1228. [PubMed: 15288701]
- Torpy DJ, Grice JE, Hockings GI, Walters MM, Crosbie GV, Jackson RV. Alprazolam blocks the naloxone-stimulated hypothalamo–pituitary–adrenal axis in man. *J Clin Endocrinol Metab*. 1993; 76:388–391. [PubMed: 8381800]
- Van Vugt DA, Piercy J, Farley AE, Reid RL, Rivest S. Luteinizing hormone secretion and corticotropin-releasing factor gene expression in the paraventricular nucleus of rhesus monkeys following cortisol synthesis inhibition. *Endocrinology*. 1997; 138:2249–2258. [PubMed: 9165008]
- West R. The multiple facets of cigarette addiction and what they mean for encouraging and helping smokers to stop. *COPD*. 2009; 6:277–283. [PubMed: 19811387]
- Wilkes S. The use of bupropion SR in cigarette smoking cessation. *Int J Chron Obstruct Pulmon Dis*. 2008; 3:45–53. [PubMed: 18488428]
- Wilson MA, Biscardi R, Smith MD, Wilson SP. Effects of benzodiazepine agonist exposure on corticotropin-releasing factor content and hormonal stress responses: divergent responses in male and ovariectomized female rats. *J Pharmacol Exp Ther*. 1996; 278:1073–1082. [PubMed: 8819488]
- Yang XM, Gorman AL, Dunn AJ, Goeders NE. Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies. *Pharmacol Biochem Behav*. 1992; 41:643–650. [PubMed: 1584846]

Fig. 1.

Effects of the combination of metyrapone (*M*) and oxazepam (*O*) and varenicline (*Var*) on IV nicotine self-administration (0.03 mg/kg/infusion) under a FR1-TO-20s schedule of reinforcement. Data are expressed as the percentage of baseline responding following vehicle pretreatment ($n=8$). Asterisk indicates $P<0.05$ vs. VEH

Fig. 2.

Effects of metyrapone (*M*) and oxazepam (*O*), alone and in combination, on IV nicotine self-administration (0.03 mg/kg/infusion) under a FR1-TO-20s schedule of reinforcement. Data are expressed as the number of nicotine infusions self-administered per session following vehicle or drug pretreatment ($n=6$). **a** Effects of 25 mg/kg metyrapone and 5 mg/kg oxazepam, alone and in combination, on nicotine self-administration. **b** Effects of 12.5 mg/kg metyrapone and 5 mg/kg oxazepam, alone and in combination, on nicotine self-administration. Asterisk indicates $P<0.05$ vs. VEH

Fig. 3.

Effects of the combination of 25 mg/kg metyrapone (*M*) and 5 mg/kg oxazepam (*O*) on IV nicotine self-administration (0.03 mg/kg/infusion) under a progressive-ratio schedule of reinforcement ($n=8$). **a** Data are expressed as the mean number of nicotine infusions per session ($\pm$ SEM). **b** Data are expressed as mean breakpoint ($\pm$ SEM). Asterisk indicates $P<0.05$ vs. VEH

Table 1

Inactive lever presses

Vehicle	Metyrapone 25 mg/kg	Oxazepam 5 mg/kg	Metyrapone 25+oxazepam 5 mg/kg	ANOVA
5.4±1.4	4.2±1.2	4.2±2.3	3.0±1.3	$F(3,12)=0.57$ $P>0.05$
Vehicle	Metyrapone 12.5 mg/kg	Oxazepam 5 mg/kg	Metyrapone 12.5+oxazepam 5 mg/kg	ANOVA
5.4±2.9	5.6±3.5	4.8±2.4	3.4±2.4	$F(3,12)=0.41$ $P>0.05$