Tolerance does not develop to the decrease in nicotine self-administration produced by repeated bupropion administration

Anthony S. Rauhut, Linda P. Dwoskin, Michael T. Bardo

[Received 25 April 2005; accepted 14 July 2005]

The atypical antidepressant bupropion has been shown to be an efficacious smoking cessation agent; however, its therapeutic mechanism of action is unknown. To further understand the mechanism by which bupropion reduces smoking, the present study determined the effect of repeated bupropion pretreatment on nicotine self-administration or sucrose-maintained responding. Rats were trained to self-administer intravenous nicotine (0.02 mg/kg infusion; Experiment 1) or to respond for sucrose pellets (45 mg each; Experiment 2) on a fixed-ratio 5 schedule. Once rats reached stable responding, bupropion (70 mg/kg, subcutaneously) or vehicle was injected 15 min before the session for 14 consecutive sessions. Bupropion acutely decreased both nicotine self-administration and sucrose-maintained responding by approximately 60%–70%. With repeated bupropion pretreatment, however, responding for nicotine decreased completely. In contrast, the bupropion-induced decrease in responding for sucrose following acute administration did not change significantly with repeated bupropion administration. These results suggest that bupropion acquired some specificity with repeated use, decreasing the intake of nicotine and producing an extinction-like pattern in nicotine self-administration. Thus the present results parallel human clinical studies with bupropion demonstrating its smoking cessation properties following repeated treatment. These results indicate that the rat nicotine self-administration paradigm is a useful animal model for assessing smoking cessation pharmacotherapies.

Introduction

The atypical antidepressant bupropion has been shown in several double-blind, placebo-controlled studies to be an effective treatment as a tobacco smoking cessation agent (Hays et al., 2001; Hurt et al., 1997; Jorenby et al., 1999). Although the pharmacological mechanism by which bupropion reduces smoking has not been elucidated, bupropion has been reported to possess both nicotinic and nonnicotinic properties in several in vitro assays. For example, using recombinant receptor expression assays, researchers showed bupropion to act as a noncompetitive antagonist at z3β2, z4β2, and z7 nicotinic receptor subtypes (Fryer & Lukas, 1999; Slemmer, Martin, & Damaj, 2000). Bupropion also has been shown to competitively inhibit nicotinic receptor subtypes mediating nicotine-evoked dopamine and norepinephrine release from striatum and hippocampus, respectively (Miller, Sumithran, & Dwoskin, 2002). Within the same concentration range in which bupropion inhibits nicotinic receptors, bupropion also inhibits the dopamine transporter and the norepinephrine transporter, having relatively low affinity for these sites compared with other selective and high-affinity transporter inhibitors (Ferris, Copper, & Maxwell, 1983).

In vivo studies have shown that bupropion dose-dependently inhibits nicotine-induced antinociception, hypoactivity, hypothermia, and convulsions in mice (Slemmer et al., 2000), suggesting that bupropion antagonizes many of the primary aversive properties of nicotine. Drug discrimination studies have found that bupropion dose-dependently generalizes to or substitutes for nicotine in rats (Wiley,
Lavecchia, Martin, & Damaj, 2002; Young & Glennon, 2002). These latter studies indicate that bupropion shares discriminative stimulus properties similar to nicotine. However, the noncompetitive nicotinic receptor antagonist mecamylamine fails to block the ability of bupropion to generalize to or substitute for nicotine, suggesting that the discriminative stimulus properties of bupropion are not nicotinic receptor mediated in rats (Wiley et al., 2002; Young & Glennon, 2002). This latter finding appears to be at odds with the results of the Slemmer et al. (2000) study, showing that bupropion is a nicotinic receptor antagonist in mice. Bondarev, Bondareva, Young, and Glennon (2004) recently suggested that differences between mice and rats in the metabolism of bupropion may account for these discrepant findings. That is, the major metabolite of bupropion, hydroxybupropion, readily accumulates in the brain and is behaviorally active in mice (Ascher et al., 1995; Damaj et al., 2004; Martin, Massol, Colin, Lacomblez, & Puech, 1990); however, it neither accumulates nor is behaviorally active in rats (Cooper et al., 1994; Suckow, Smith, Perumal, & Cooper, 1986).

In vivo studies examining the effect of bupropion on nicotine self-administration in rats have produced mixed results. Acute bupropion pretreatment produces a biphasic dose-response function on nicotine self-administration, with low doses (9–15 mg/kg) of bupropion increasing and high doses (78 mg/kg) of bupropion decreasing the number of nicotine infusions self-administered (Rauhut, Neugebauer, Dwoskin, & Bardo, 2003). Interestingly, the psychostimulant methamphetamine produces a similar biphasic dose-response function when administered prior to nicotine self-administration (Rauhut et al., 2003). Shoab, Sidhpura, and Shafait (2003) also reported that bupropion (30 mg/kg) increases nicotine self-administration in rats. In contrast, acute bupropion pretreatment has been reported by others to dose-dependently decrease nicotine self-administration using either fixed-ratio (Bruijnzeel & Markou, 2003; Glick, Maisonneuve, & Kitchen, 2002; Maisonneuve & Glick, 2003) or progressive-ratio (Bruijnzeel & Markou, 2003) schedules of reinforcement. The reason for the discrepancies among laboratories is unlikely due to the operant schedules utilized, nicotine doses chosen, or bupropion-pretreatment regimen selected, as these parameters were similar among laboratories. Bruijnzeel and Markou (2003) suggested that rat strain differences may account for the discrepancies, given that bupropion has been shown to produce differences between mouse strains with regard to its degree of antidepressant effect (Shanks & Anisman, 1989). Consistent with the above-noted studies showing that bupropion produces a monophasic, dose-dependent decrease in nicotine self-administration, Cryan, Bruijnzeel, Skjei, and Markou (2003) also found that bupropion dose-dependently attenuated nicotine-induced decreases in brain stimulation reward thresholds in rats. Taken together, these latter studies indicate that bupropion attenuates the reinforcing effect of nicotine.

As a tobacco smoking cessation therapy, bupropion is administered repeatedly (150–300 mg/d) and is reported to be well tolerated (Hays et al., 2001; Hurt et al., 1997; Jorenby et al., 1999). Shoab et al. (2003) reported that repeated pretreatment with bupropion (30 mg/kg) for 28 days increased nicotine self-administration in rats, suggesting that tolerance does not develop to the rate-increasing effects of bupropion on nicotine self-administration. Moreover, Shoab et al. (2003) found that on termination of repeated bupropion pretreatment, nicotine self-administration returned to baseline levels, suggesting little carryover effect.

Surprisingly, the effect of repeated pretreatment with bupropion at a dose that decreases nicotine self-administration has not been determined. Thus the present study determined the effect of repeated bupropion pretreatment using a dose (70 mg/kg) shown previously to decrease nicotine self-administration by approximately 50% (Rauhut et al., 2003). To assess the specificity of the effect of repeated bupropion pretreatment on nicotine self-administration, we also determined the ability of repeated bupropion treatment to decrease sucrose-maintained responding.

Method

Subjects

Male Sprague-Dawley drug-naïve rats (200–225 g), obtained from Harlan Industries (Indianapolis, Indiana), were used in all experiments. Rats had unlimited access to food and water in the home cage, except as noted. Rats were maintained on a 14:10 h light-dark cycle in which the lights came on at 0600 h and went off at 2000 h. All experiments described in this report occurred during the light phase of the cycle. Rats were acclimated to the animal colony and were briefly handled daily prior to the start of the experiment. The Institutional Animal Care and Use Committee of the University of Kentucky approved the experiments described herein. The experiments conformed to the guidelines established by the 1996 edition of the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

We used six operant chambers (ENV-001; Med Associates, St. Albans, Vermont) housed in sound-attenuated chambers. The end walls of these chambers were aluminum, and the front and back
walls were clear Plexiglas. The floor consisted of 18 stainless steel rods (4.8 mm) placed 1.6 cm apart. Located in the bottom center of one of the end walls was an opening (5 × 4.2 cm) for a recessed food tray. Located on either side of the recessed food tray was a response lever. A 28-V white cue light was located 6 cm above each response lever. All stimulus and response events were controlled by a personal computer located in the room.

**Drugs**

St(-)-Nicotine di-tartrate (RBI, Natick, Massachusetts) was prepared in a physiological saline solution, to which NaOH was added to obtain a pH of 7.4. (±)-Bupropion HCl (provided by John Reinhard, GlaxoWellcome, Research Triangle Park, North Carolina) was prepared in phosphate-buffered saline (PBS) and administered in a volume of 1 ml/kg body weight. Nicotine was administered intravenously, and the dose was expressed as the free-base weight. Bupropion was administered subcutaneously, and the dose was expressed as the salt weight. Ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa) and diazepam (Abbott, North Chicago, Illinois) were administered intraperitoneally in a volume of 1 ml/kg body weight. Doses of ketamine and diazepam were expressed as their salt weights.

**Surgery**

Rats were anesthetized by an injection of ketamine (80 mg/kg, intraperitoneally) and diazepam (5 mg/kg, intraperitoneally), and an indwelling silastic catheter was inserted into the jugular vein. The free end of the catheter exited through the skin and was secured to an acrylic head mount attached to the skull. A silastic leash was used to attach the head mount to an infusion pump.

**General procedures**

**Nicotine self-administration.** The nicotine self-administration procedure was similar to that described previously (Bardo, Green, Crooks, & Dwoskin, 1999). Rats were deprived to 85% of their ad libitum body weights by restricting their intake of rat chow to 5–10 g/d for 7 days. Subsequently, rats were trained on a fixed-ratio (FR) 1 schedule of sucrose reinforcement for a 15-min period (in the dark) and were required to earn 20 reinforcers. The FR 1 schedule of reinforcement was increased to FR 2 (1 session) and then to FR 5 (3 sessions). After training for sucrose reinforcement, rats were allowed ad libitum access to food and then were implanted with an indwelling jugular catheter. Following recovery from surgery, rats were food restricted (~20 g/d) and reintroduced to the operant chamber (in the dark) for a daily 60-min session in which responses made on one lever (active) were recorded and followed by intravenous infusions of nicotine (0.02 mg/kg/infusion, 60 μl infusion delivered over 3.45 sec). Responses made on the other lever (inactive) were recorded but had no scheduled consequence. Completion of the FR requirement resulted in the simultaneous activation of the infusion pump and the cue lights (located above each lever), which signaled a 20-sec timeout period during which lever pressing had no scheduled consequence. The cue lights remained illuminated during the 20-sec timeout period. The FR 1 schedule of reinforcement was increased to FR 2, FR 3, FR 4, and FR 5, contingent on the following criteria: a minimum of five infusions and 2:1 (active:inactive) response ratio. Rats were trained to lever press for infusions of nicotine until responding stabilized on an FR 5 schedule of reinforcement.

**Sucrose-maintained responding.** For sucrose-maintained responding, rats were reduced to 85% of their ad libitum weight. Training procedures were similar to those described in the nicotine self-administration experiments, except that rats did not undergo surgery, they earned sucrose pellets (45 mg each pellet) instead of nicotine infusions, and delivery of sucrose pellets was not accompanied by activation of cues lights or 20-sec timeout periods. For these experiments, the session duration was 15 min to avoid a decrease in responding due to satiation within the session. Rats were trained to lever press for sucrose pellets until responding stabilized on an FR 5 schedule of reinforcement.

**Specific experiments**

The ability of repeated bupropion pretreatment to decrease nicotine self-administration (Experiment 1) or sucrose-maintained responding (Experiment 2) was assessed. Once rats reached a stable rate of responding, they received a subcutaneous injection of either vehicle (PBS; control) or bupropion (70 mg/kg) 15 min prior to the nicotine self-administration or sucrose-maintained responding session for 14 consecutive daily sessions. The dose of bupropion was chosen based on previous research in our laboratory showing that acute pretreatment with this dose decreased nicotine self-administration by approximately 50% (Rauhut et al., 2003). For the nicotine self-administration experiment, the initial pretreatment was initiated when the following criteria were met: (1) at least five nicotine infusions per session, (2) less than 20% variability in responding for two consecutive sessions, and (3) a minimum of 2:1 (active:inactive) response ratio. Criteria for the initial
pretreatment in the sucrose-maintained responding experiment were similar to the nicotine self-administration experiment, except that rats were required to earn 10 sucrose pellets for two consecutive sessions. In both experiments, following the last bupropion-pretreatment session, all rats were pretreated with vehicle prior to the session (Session 15).

Data analyses
In Experiments 1 and 2, three-way mixed-design analyses of variance (ANOVAs) were conducted with drug (bupropion vs. vehicle) as a between-group factor, and lever (active vs. inactive) and session as within-subject factors, followed by point-by-point t tests comparing the bupropion pretreatment group and the vehicle control group on each session (SPSS version 9.0). Post hoc contrasts involved either independent or correlated t tests for between- or within-subject analyses, respectively. Statistical significance was indicated by p<.05.

Results
Experiment 1
A three-way ANOVA revealed significant main effects of drug, F(1, 9)=67.9, p<.001, and session, F(15, 135)=3.7, p<.001, as well as a significant drug x session interaction, F(15, 135)=5.4, p<.001. Post hoc contrasts revealed that during the first 60-min session, bupropion (n=5) decreased nicotine self-administration by approximately 70% relative to the control (n=6), t(9)=4.6, p<.001 (Figure 1, left panel). Moreover, bupropion continued to decrease responding for nicotine compared with control during the remaining 13 drug-pretreatment sessions, t values (9)>4.0, p values<.01, suggesting that tolerance did not develop to this effect of bupropion. Additionally, in the bupropion-pretreated rats, responding for nicotine was less on Session 14 than on Session 1, correlated t (4)=2.7, p<.05, indicating that the rate-decreasing effect of bupropion was enhanced with repeated pretreatment. Once bupropion pretreatment was terminated, responding for nicotine remained suppressed during Session 15, t(9)=4.6, p<.01. Because the effect of repeated bupropion pretreatment on sucrose-maintained responding was determined (Experiment 2), and because sucrose-maintained responding sessions were only 15 min in duration, the first 15 min of the 60-min nicotine self-administration session also was analyzed. This analysis revealed similar patterns to those observed in the 60-min sessions (Figure 1, right panel).

![Figure 1](image-url)

**Figure 1.** Number of responses emitted (mean ± SEM) on the active and inactive levers during three phases of Experiment 1: baseline, drug pretreatment, and vehicle pretreatment. During the drug-pretreatment phase, rats received a subcutaneous injection of either bupropion (70 mg/kg) or vehicle (control) 15 min prior to a 60-min nicotine self-administration session on a fixed-ratio 5 schedule of reinforcement (left panel). For comparison to the sucrose-maintained responding results, data are also plotted for the first 15 min of the 60-min session (right panel). Asterisks (*) denote a significant difference in responding between bupropion-pretreated rats and control rats. Pound (#) symbols denote a decrease in responding on a particular session for bupropion-pretreated rats compared with the first session of the drug-pretreatment phase. For clarity, asterisks denoting group differences in responding on the inactive lever have been omitted. All p values<.05. N=5–6 rats per group.
The three-way ANOVA also revealed a significant drug × lever × session interaction, $F(15, 135) = 4.2$, $p < .001$. Post hoc contrasts revealed that responding on the inactive lever was decreased on Sessions 3, 5, 6, 7, 8, 9, 10, 11, 12, and 14 in bupropion-pretreated rats relative to control rats, $t$ values $(9) > 2.3$, $p$ values $< .05$. A similar pattern was observed when the data for the first 15 min of the session were analyzed.

**Experiment 2**

A three-way ANOVA on the data for sucrose-maintained responding revealed significant main effects of group, $F(1, 9) = 35.8$, $p < .001$, and session, $F(15, 135) = 3.9$, $p < .001$, as well as a significant group × session interaction, $F(15, 135) = 5.9$, $p < .001$. Post hoc contrasts revealed that bupropion $(n = 6)$ initially decreased sucrose-maintained responding by approximately 60% relative to control $(n = 6)$, $t(10) = 3.8$, $p < .01$ (Figure 2). Bupropion decreased responding for sucrose during the remaining 13 sessions relative to control, $t$ values $(10) > 2.3$, $p$ values $< .01$. In the bupropion-pretreated group, responding for sucrose on Session 14 did not differ from Session 1, $t(5) = 1.4$, $p > .1$, indicating that the effect of bupropion on sucrose-maintained responding was not altered with repeated pretreatment. When the bupropion pretreatment was terminated and replaced with vehicle, responding was decreased on Session 15, $t(10) = 3.2$, $p < .01$.

Similar to Experiment 1, a significant drug × lever × session interaction was found, $F(15, 135) = 5.6$, $p < .001$. Post hoc contrasts revealed that responding on the inactive lever was decreased in the bupropion-pretreated rats on Sessions 4 and 5 compared with the control rats, $t$ values $(10) > 2.4$, $p$ values $< .05$. These significant differences are not visually apparent in Figure 2 owing to the scale used to plot the number of responses on each lever.

**Discussion**

During the first self-administration session, bupropion (70 mg/kg) decreased nicotine self-administration by about 70%, replicating previous work (Bruijnzeel & Markou, 2003; Glick et al., 2002; Maisonneuve & Glick, 2003; Rauhut et al., 2003). Moreover, bupropion continued to decrease nicotine self-administration following repeated pretreatment, indicating that tolerance does not develop to the rate-decreasing effect of bupropion. Rather, the bupropion-induced decrease in nicotine self-administration was enhanced by repeated pretreatment, perhaps reflecting extinction of nicotine self-administration behavior related to the nicotinic antagonist properties of bupropion (Fryer & Lukas, 1999; Miller et al., 2002; Slemmer et al., 2000). In support of this interpretation, mecamylamine pretreatment has been shown to produce extinction of nicotine self-administration (Donny, Caggiula, Knopf, & Brown, 1995; Watkins, Epping-Jordan, Koob, & Markou, 1999). However, because the effect of repeated mecamylamine pretreatment was not examined in the present study, this interpretation is speculative.

The enhanced ability of bupropion to decrease nicotine self-administration across repeated injections parallels clinical studies showing that the therapeutic effect of bupropion as a tobacco smoking cessation agent is enhanced by repeated use (Hays et al., 2001; Hurt et al., 1997; Jorenby et al., 1999). In studies demonstrating efficacy of bupropion as a tobacco smoking cessation agent, treatment began 8 days prior to a “quit date,” such that patients attained steady-state plasma levels of bupropion (Ferris et al., 1983; Hays et al., 2001; Hurt et al., 1997; Jorenby et al., 1999). In the present experiment, termination of bupropion pretreatment continued to decrease nicotine self-administration, suggesting a carryover effect of bupropion consistent with the
relatively slow elimination of the drug (Suckow et al., 1986). Thus the effect of repeated bupropion administration in the intravenous nicotine self-administration model parallels clinical findings in smoking cessation studies.

Results of the present study showed that bupropion decreased sucrose-maintained responding by approximately 60%, also replicating previous work (Rauhut et al., 2003). Brujinzeel and Markou (2003) also found that bupropion (40 mg/kg) tended to decrease food-maintained responding, but this effect failed to reach statistical significance. Similar to the results with nicotine self-administration, tolerance did not develop to the rate-decreasing effect of bupropion on sucrose-maintained responding (despite procedural differences in the two preparations), and the decrease in responding was apparent following the termination of bupropion pretreatment. However, in contrast to the results with nicotine self-administration, the 60% decrease in sucrose-maintained responding was unaltered, rather than enhanced, across repeated bupropion pretreatment sessions. The failure of repeated bupropion pretreatment to produce an extinction-like pattern of sucrose-maintained responding, similar to its effect on nicotine self-administration, provides further support for the idea that bupropion decreases nicotine self-administration, at least in part, via its interaction with nicotinic receptors, acquiring some specificity with continued use. Moreover, acute bupropion (75 mg/kg) pretreatment has been reported to decrease food consumption in a non-operant task (Zarrindast & Hosseini-Nia, 1988), suggesting that bupropion has anorectic properties. Indeed, bupropion produces weight loss in clinically obese populations (Anderson et al., 2002) and attenuates weight gain associated with depression (Fava, 2000; Jain et al., 2002) and smoking cessation (Hays et al., 2001; Hurt et al., 1997). Thus the bupropion-induced decrease in sucrose-maintained responding, as observed in the present report, may be due to the anorectic properties of bupropion and parallels clinical findings.

However, the observed differential effects of bupropion on nicotine self-administration and sucrose-maintained responding are complicated by differences in response rates. That is, the failure of bupropion to more effectively decrease sucrose-maintained responding, similar to its effects on nicotine self-administration, may be due to differences in response rates engendered by nicotine and sucrose. However, it has been observed that drugs more readily decrease high rates compared with low rates of operant responding (Phillips, Willner, Sampson, Nunn, & Muscat, 1991). Thus, if the differential effects of bupropion on nicotine self-administration and sucrose-maintained responding were due to differences in response rates, then bupropion would be predicted to more effectively decrease sucrose-maintained responding compared with nicotine self-administration. Because the opposite result was observed, it is unlikely that the differential effects of bupropion on nicotine self-administration and sucrose-maintained responding were simply due to differences in response rates.

The present experiments utilized a relatively high bupropion dose. As such, nonspecific factors (e.g., motor impairment) may have contributed to the complete elimination of nicotine self-administration that developed across the repeated bupropion regimen. Indeed, in the nicotine self-administration experiment, bupropion decreased responding on the inactive lever during many of the nicotine self-administration sessions. However, under the same repeated bupropion regimen, rats responding for sucrose continued to emit about 100 responses and earn 20 sucrose pellets on average in 15 min following 14 days of bupropion pretreatment (see Figure 2). These latter results demonstrate that rats were able to respond under the repeated high-dose bupropion regimen, and that the bupropion-induced decrease in nicotine self-administration was not due solely to response impairment. In the end, however, it is difficult to assess the contribution of nonspecific factors, because the rats were not observed directly during the nicotine self-administration or sucrose-maintained responding sessions.

The contribution of the metabolites of bupropion to the decrease in nicotine self-administration cannot be excluded. In mice and humans, bupropion is readily metabolized to hydroxybupropion, which is behaviorally active and has been suggested to contribute largely to the antidepressant (Ascher et al. 1995; Martin et al. 1990) and smoking cessation (Damaj et al., 2004) effects of bupropion. In fact, the 2S,3S isomer of hydroxybupropion has been shown to more potently inhibit the human α4β2 nicotinic receptor, compared with the 2S,3R isomer or racemic mixture of bupropion (Damaj et al., 2004). In rats, however, hydroxybupropion does not accumulate in the brain in significant levels (Suckow et al. 1986), and it contributes little to the behavioral effects of bupropion (Cooper et al. 1994). Thus the observed decrease in nicotine self-administration following repeated bupropion pretreatment in the present experiment is not likely to be due to hydroxybupropion.

In summary, the present experiments revealed that a high bupropion dose initially decreased both nicotine self-administration and sucrose-maintained responding. With repeated pretreatment, bupropion decreased nicotine self-administration even further; however, the decrease of sucrose-maintained responding was unaltered. Taken together, these results suggest that
bupropion acquires some pharmacological specificity with continued use. Moreover, bupropion produced an extinction-like pattern on nicotine self-administration, perhaps contributing to its efficacy as a smoking cessation agent (Hays et al., 2001; Hurt et al., 1997; Jorenby et al., 1999).

Acknowledgments

This research was supported by the Pharmacia Corporation (Kalamazoo, Michigan). Anthony S. Rauhut was supported by a National Institutes of Health National Research Service Award (T32 DA07304) from the National Institute on Drug Abuse. The authors thank Laura Fenton, Thomas Green, and Brenna Shortridge for their excellent technical assistance.

References


