Original Investigation

Bupropion and its Main Metabolite Reverse Nicotine Chronic Tolerance in the Mouse

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Abstract

Introduction: Although the antidepressant bupropion is prescribed to aid in smoking cessation, little is known concerning its mechanisms of action in this regard. One factor that might influence quit success is nicotine tolerance, which could promote high levels of nicotine intake in order to maintain nicotine's subjective effects (thereby making attempts to reduce smoking more difficult).

Methods: To explore whether bupropion and its active hydroxymetabolite modulate nicotine tolerance, mice were injected for 14 days with saline or nicotine. On Day 14, animals received saline, (2S,3S)-hydroxybupropion, or bupropion at different doses. On Day 15, mice were assayed on test day for nicotine-induced analgesia and hypothermia.

Results: Animals chronically injected with saline + nicotine developed tolerance to nicotine's effects in both assays. Administration of bupropion and (2S,3S)-hydroxybupropion dose-dependently reversed chronic nicotine tolerance.

Conclusions: These results indicate that bupropion's ability to promote smoking cessation may be partly due to its attenuation of nicotine tolerance since both measured responses of nicotine (antinociception and hypothermia) are mediated to a large extent by neuronal $\alpha 4\beta 2^*$ nicotine receptors.

Introduction

Bupropion is an atypical antidepressant that is also prescribed as a smoking-cessation aid. However, the mechanisms through which bupropion acts to promote quit attempts has not been totally clarified. For example, bupropion has been shown to alleviate withdrawal signs in humans (Shiffman et al., 2000), which may be due to its modulatory actions on dopaminergic and noradrenergic systems (Cooper et al., 1994). Specifically, its inhibition of transporter function results in increased extracellular dopamine and norepinephrine concentrations, which may substitute for nicotine-evoked release of these neurotransmitters

and alleviate withdrawal during nicotine abstinence (Ascher et al., 1995). In this manner, bupropion seems to indirectly act like a nicotine compound.

However, studies also indicate that bupropion is a relatively potent, noncompetitive antagonist at nicotinic acetylcholine receptors (Slemmer, Martin, & Damaj, 2000). In addition, bupropion blocks nicotine-induced antinociception, hyperactivity, hypothermia, and convulsions (Slemmer et al., 2000). Further, some reports have demonstrated that bupropion attenuates nicotine self-administration in the rat (Bruijnzeel & Markou, 2003; Glick, Maisonneuve, & Kitchen, 2002). However, others have found that bupropion enhances nicotine's effects in the self-administration design (Rauhut, Neugebauer, Dwoskin, & Bardo, 2003), indicating that bupropion's effects on nicotine reinforcement are mixed. Therefore, bupropion seems to act similarly to nicotine in some dependence behaviors but as a nicotinic antagonist in others. Furthermore, bupropion is extensively metabolized to (2R,3R)- and (2S,3S)-hydroxybupropion, (R,R)- and (S,S)-threohydrobupropion, and (R,S)- and (S,R)-erythrohydrobupropion in humans and mice (Cooper et al., 1994). Bupropion administration is typically initiated at least 7 days prior to the quit attempt (George & O'Malley, 2004), so it is unclear whether its efficacy is due the blockade of nicotine's effects prior to the quit attempt and/or to its ability to alleviate symptoms of nicotine withdrawal during cessation. For example, acute and chronic bupropion administration reversed and prevented, respectively, the anhedonic aspects of nicotine withdrawal in the rat (Cryan, Bruijnzeel, Skjei, & Markou, 2003; Paterson, Balfour, & Markou, 2007) and mice (Damaj et al., 2010). Another possible mechanism is that bupropion may prevent the development of nicotine tolerance, which may allow smokers to gradually reduce their levels of smoking (as opposed to abruptly going "cold turkey"). In addition, blockade of nicotine tolerance may make nicotine replacement therapies more effective. This might explain why coadministration of bupropion and nicotine replacement therapy during withdrawal has been shown, in at least one report, to be more effective than either treatment alone in maintaining smoking abstinence (Gold, Rubey, & Harvey, 2002).

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Although there are no published reports on bupropion's ability to prevent the development of nicotine tolerance, animal models can allow for an examination of this issue. Specifically, mice or rats chronically exposed to nicotine demonstrated reduced responsivity to acute nicotine such as antinociception (Damaj, Welch, & Martin, 1996; Grabus et al., 2005; McCallum et al., 1999) and hypothermia (Grabus et al., 2005; Robinson, Grun, Pauly, & Collins, 1996). Given the extensive metabolism of bupropion to hydroxybupropion in humans and mice, its long-half life, its neurobiological activity, the (2S,3S)-hydroxybupropion isomer may play an important part in the mechanism of action of bupropion in nicotine dependence. Therefore, the present experiment explored whether different doses of bupropion and its main metabolite (2S,3S)-hydroxybupropion could reverse chronic tolerance to nicotine-induced antinociception and hypothermia in mice.

Methods

Subjects

Subjects were experimentally naïve, male Institute for Cancer Research mice (Harlan Laboratories, Indianapolis, IN). They were housed in a 21 °C humidity-controlled Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility with food and water available *ad libitum*. The rooms were on a 12-hr light/dark cycle (lights on at 7:00 a.m.). Animals were about ten weeks of age and weighed approximately 25–30 g at the start of the experiment. All experiments were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and in accordance with the National Institutes of Health Guide for Animal Care and Use.

Drugs

(–)-Nicotine hydrogen tartrate salt [(–)-1-methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt] was purchased from Sigma-Aldrich Inc. (St. Louis, MO). Bupropion HCl was purchased from Research Biochemical, Inc. (Natick, MA). (+)-(2S,3S)-hydroxybupropion tartrate was synthesized using reported methods (Fang et al., 2000). All drugs were dissolved in physiological saline (0.9% sodium chloride) and given in a total volume of 1 ml/100 g body weight for subcutaneous (s.c.) injections. All doses are expressed as the free base of the drug. Nicotine doses were based on published articles on nicotine-induced antinociception and hypothermia in the mouse (Damaj et al., 1996; Grabus et al., 2005). All doses were expressed as the free base of the drug.

Procedure

Experimental subjects were injected twice daily (8:00 a.m. and 5:00 p.m.) for 14 days with saline or nicotine (2 mg/kg; $n = 6$ –8 per group). On Day 14 (5:00 p.m.) after the last dose of nicotine or saline, different groups of mice received s.c. saline, (2S,3S)-hydroxybupropion (1, or 5 mg/kg) or bupropion (1, 10, or 20 mg/kg; $n = 6$ –8 per group). On Day 15 (5:00 p.m.), a control response was determined for the antinociception and hypothermia assays for these animals (i.e., prior to any injection). Following these determinations, animals were injected with s.c. 2.5 mg/kg nicotine and 5 min or 20 min later they were tested for antinociception and hypothermia, respectively. Control

animals received s.c. saline + saline, (2S,3S)-hydroxybupropion or bupropion + saline on Day 14 (5:00 p.m.; $n = 6$ –8 per group). The specific tests were as follows:

Tail-Flick

Antinociception was assessed by the tail-flick method of D'Amour and Smith (1941; Thermojust Apparatus). To minimize tissue damage, a maximum latency of 10 s was imposed. Antinociceptive response was calculated as percent maximum possible effect (%MPE, where %MPE = $[(\text{test} - \text{control}) / (10 - \text{control})] \times 100$).

Body Temperature

Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Δ °C or the difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory was between 21 and 24 °C.

Data Analysis

Significant overall one-way analysis of variance (ANOVA) was followed by post-hoc comparisons (Fisher's protected least significant test). All statements of statistical significance are based on $p < .05$.

Results

Tail-Flick Antinociception

Bupropion dose-dependently reversed chronic tolerance to nicotine-induced antinociception in the tail-flick test ($F(6, 41) = 83.7$; $p < .0001$; Figure 1A). Post-hoc tests indicated that, compared with saline + saline controls, animals chronically injected with saline + nicotine demonstrated a loss of nicotine-induced antinociception ($p < .0001$). Compared with the saline + nicotine group, bupropion at 20 mg/kg totally reversed nicotine tolerance in the tail-flick test ($p = .004$) but not 1 mg/kg ($p < .7825$).

Similar to bupropion, the (2S,3S)-hydroxyisomer reversed the chronic tolerance to nicotine in a dose-related manner ($F(5, 35) = 36.09$; $p < .0001$; Figure 1B). Post-hoc tests indicated that, compared with saline + saline controls, animals chronically injected with saline + nicotine demonstrated a loss of nicotine-induced antinociception ($p < .0001$). Compared with the saline + nicotine group, (2S,3S)-hydroxybupropion at 5 mg/kg totally reversed nicotine tolerance in the tail-flick test ($p = .004$) but not 1 mg/kg ($p < .635$). Therefore, tolerance to nicotine antinociceptive effects was dose-dependently reversed by bupropion and (2S,3S)-hydroxybupropion.

Body Temperature

Bupropion dose-dependently reversed chronic tolerance to nicotine-induced hypothermia ($F(6, 41) = 37.53$; $p < .0001$; Figure 2A). Post-hoc tests indicated that, compared with saline + saline controls, animals chronically injected with saline + nicotine demonstrated a loss of nicotine-induced antinociception ($p < .001$). Compared with the saline + nicotine group, bupropion at 20 mg/kg totally reversed nicotine tolerance in the tail-flick test ($p < .004$) but not 1 mg/kg ($p < .5635$).

Figure 1. Mice were injected (subcutaneous (s.c.), twice a day) for 14 days with saline or nicotine (2 mg/kg; $n = 6-8$ per group). Bupropion (1 [light gray], 10 [dark gray] or 20 [black] mg/kg) or saline (open) were given at Day 14. On Day 15, all animals were injected with either saline (Panel A left) nicotine (2.5 mg/kg, s.c.) and tested for tail-flick % analgesia (Panel A right) and $\Delta^\circ\text{C}$ or the difference in body temperature (Panel B). Results are presented as $M \pm \text{SEM}$ for each measure. * $p < .05$ from Saline challenge/saline group and # $p < .05$ from Nicotine challenge/saline group.

Similar to bupropion, the (2S,3S)-hydroxyisomer reversed the chronic tolerance to nicotine in a dose-related manner ($F(5, 35) = 64.5$; $p < .0001$; Figure 2B). Post-hoc tests indicated that, compared with saline + saline controls, animals chronically injected with saline + nicotine demonstrated a loss of nicotine-induced hypothermia ($p < .0001$). Compared with the saline + nicotine group, (2S,3S)-hydroxybupropion at 5 mg/kg totally reversed nicotine tolerance ($p < .003$) but not 1 mg/kg ($p < .355$). Therefore, tolerance to nicotine hypothermia was dose-dependently reversed by bupropion and (2S,3S)-hydroxybupropion.

Discussion

Clinical studies with bupropion provided evidence for withdrawal amelioration in smokers and an attenuation of nicotine rewarding effects (For review see Paterson, 2009). However, due to the multidimensional nature of tobacco dependence, it is still not very clear how bupropion was achieving favorable outcomes as a smoking cessation agent. Our results indicate that bupropion and its active metabolite (2S,3S)-hydroxybupropion reversed chronic tolerance to nicotine in animals. This observed effect of bupropion may be important to its efficacy as an aid to smoking cessation. Chronic smokers may develop tolerance to certain nicotine-mediated effects, which would cause them

to increase their nicotine intake in order to maintain a certain subjective sensation. By attenuating nicotine tolerance, bupropion may make this compensatory increase unnecessary. Smokers could therefore gradually reduce their nicotine intake without any negative side effects (e.g., withdrawal). In addition, administration of bupropion prior to a quit attempt may make nicotine replacement therapies more effective (i.e., through preventing nicotine tolerance, thereby making nicotine replacement therapies more potent).

Different mechanisms can explain the reversal of nicotine chronic tolerance by bupropion and (2S,3S)-hydroxybupropion. On one hand, they could be reversing the tolerance by acting as noncompetitive nicotinic antagonists. This is consistent with bupropion and (2S,3S)-hydroxybupropion's *in vivo* antagonist-like effects in blocking nicotine-induced antinociception and hypothermia and their *in vitro* noncompetitive antagonistic properties at $\alpha 4\beta 2$ and $\alpha 3\beta 4$ neuronal nicotinic acetylcholine receptors (Damaj et al., 2004; Slemmer et al., 2000). This mechanism is consistent with an earlier study reporting that mecamylamine, a noncompetitive nicotinic antagonist, blocks the development of tolerance to nicotine-induced antinociception in rats (McCallum et al., 1999). It is interest to note that both measured responses of nicotine (antinociception and hypothermia) are mediated to a large extent by neuronal $\alpha 4\beta 2^*$ nicotine receptors (Marubio et al., 1999; Tritto et al., 2004)

Figure 2. Mice were injected (subcutaneous (s.c.), twice a day) for 14 days with saline or nicotine (2 mg/kg; $n = 6-8$ per group). (2S,3S)-Hydroxybupropion (1 [gray] or 5 [black] mg/kg) or saline (open) were given at Day 14. On Day 15, all animals were injected with either saline (Panel A left) nicotine (2.5 mg/kg, s.c.) and tested for tail-flick % analgesia (Panel A right) and $\Delta^{\circ}\text{C}$ or the difference in body temperature (Panel B). Results are presented as $M \pm \text{SEM}$ for each measure. * $p < .05$ from Saline challenge/saline group and # $p < .05$ from Nicotine challenge/saline group.

which play an important role in nicotine reward as measured by conditioned place preference (Walters, Brown, Changeux, Martin, & Damaj, 2006) and iv self-administration (Picciotto et al., 1998). These subtypes were also shown to play an important role in nicotine withdrawal (Jackson, Martin, Changeux, & Damaj, 2008). Although measuring antinociceptive and hypothermic responses may not directly relate to nicotine dependence, it would provide a quantitative index of certain aspects that have some clinical relevance. For example, antinociceptive responses would be considered a measure of relief of discomfort or irritation. However, the importance of measure acute effects of nicotine lies in the fact that pathways and mechanisms leading to more relevant complex behavioral effects of nicotine are initiated by binding to relevant nicotinic acetylcholine receptor subtypes in the brain.

On the other hand, nicotine could produce its effects on acute pain and hypothermia via downstream neurotransmitters like epinephrine. Indeed, antinociception produced by s.c. nicotine is mediated via a number of neuronal sites including α -2 adrenergic but not dopaminergic mechanisms (Damaj & Martin, 1993; Damaj, Welch, & Martin, 1994; Rogers & Iwamoto, 1993). The tolerance to chronic nicotine might be due to adaptive changes to pre- or postsynaptic noradrenergic mechanisms. The ability of bupropion and its metabolite to mitigate such adaptive changes by enhancing norepinephrine release could explain the reversal of nicotine chronic tolerance. The fact that (2S,3S)-hydroxybupropion was more potent than

bupropion in reversing the tolerance, correlates with its higher potency on norepinephrine uptake (Damaj et al., 2004).

Our findings with (2S,3S)-hydroxybupropion using a mouse model of nicotine chronic tolerance support the hypothesis that bupropion's utility as a pharmacological treatment of nicotine dependence reflects actions of bupropion and/or its hydroxymetabolites. The concentrations of hydroxybupropion isomers present in cerebrospinal fluid are six times greater than those of the parent bupropion (Cooper et al., 1994). Indeed, bupropion metabolism is closer in humans and mice than humans and rats. Specifically, there is much lower formation and more rapid elimination of hydroxybupropion in rats versus humans (Suckow, Smith, Perumal, & Cooper, 1986; Welch, Lai, & Schroeder, 1987).

In summary, bupropion's ability to aid in smoking cessation may be a result of both its nicotine-like effects as well as its nicotinic antagonist properties. Specifically, bupropion may attenuate certain nicotine-mediated effects (such as the development of tolerance) while acting as a substitute for nicotine in other measures. As mentioned previously, bupropion's inhibition of transporter function increases extracellular levels of dopamine and norepinephrine, which may substitute for nicotine-evoked release of these neurotransmitters and alleviate withdrawal during nicotine abstinence (Ascher et al., 1995). Consistent with this, bupropion has been shown to alleviate specific withdrawal signs in mice (Damaj et al., 2010) and

humans, including irritability, depression, and concentration difficulties. In addition, we reported that bupropion substitutes for nicotine in the rat discrimination model (Wiley, Lavecchia, Martin, & Damaj, 2002). Therefore, it appears that bupropion acts as a nicotinic-like compound in some measures, but not others.

The results from the present experiment indicate that bupropion attenuates tolerance to certain nicotine-mediated effects. Therefore, bupropion's efficacy as a smoking-cessation aid may be related to both its nicotine-like and its nicotinic antagonist effects.

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Declaration of Interests

None declared.

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