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ABSTRACT

Bupropion, an efficacious antidepressant and smoking cessation agent, inhibits dopamine and norepinephrine transporters (DAT and NET, respectively). Recently, bupropion has been reported to noncompetitively inhibit $\alpha_3\beta_2$, $\alpha_3\beta_4$, and $\alpha_4\beta_2$ nicotinic acetylcholine receptors (nAChRs) expressed in Xenopus oocytes or established cell lines. The present study evaluated bupropion-induced inhibition of native $\alpha_3\beta_2$- and $\alpha_3\beta_4$-nAChRs using functional neurotransmitter release assays, nicotine-evoked $[^3]H$overflow from superfused rat striatal slices preloaded with $[^3]H$dopamine ($[^3]H$DA), and nicotine-evoked $[^3]H$overflow from hippocampal slices preloaded with $[^3]H$norepinephrine ($[^3]H$NE). The mechanism of inhibition was evaluated using Schild analysis. To eliminate the interaction of bupropion with DAT or NET, nomifensine or desipramine, respectively, was included in the superfusion buffer. A high bupropion concentration (100 $\mu$M) elicited intrinsic activity in the $[^3]H$DA release assay. However, none of the concentrations (1 nM–100 $\mu$M) examined evoked $[^3]H$NE overflow and, thus, were without intrinsic activity in this assay. Moreover, bupropion inhibited both nicotine-evoked $[^3]H$DA overflow (IC$_{50}$ = 1.27 $\mu$M) and nicotine-evoked $[^3]H$NE overflow (IC$_{50}$ = 323 nM) at bupropion concentrations well below those eliciting intrinsic activity. Results from Schild analyses suggest that bupropion competitively inhibits nicotine-evoked $[^3]H$DA overflow, whereas evidence for receptor reserve was obtained upon assessment of bupropion inhibition of nicotine-evoked $[^3]H$NE overflow. Thus, bupropion acts as an antagonist at $\alpha_3\beta_2$- and $\alpha_3\beta_4$-nAChRs in rat striatum and hippocampus, respectively, across the same concentration range that inhibits DAT and NET function. The combination of nAChR and transporter inhibition produced by bupropion may contribute to its clinical efficacy as a smoking cessation agent.

Clinical studies have revealed a strong correlation between the incidence of tobacco smoking and mood disorders (Glassman et al., 1990; Pomerleau et al., 2000). Individuals with clinical depression are more likely to be tobacco smokers, dependent on nicotine, and to experience difficulty quitting with greater withdrawal symptoms upon cessation (Covey et al., 1997; Covey, 1999). Smokers undergoing cessation experience symptoms of depression, occurring more frequently among smokers with a history of major depression (Covey et al., 1997). The antidepressant, bupropion, has therapeutic benefit as a smoking cessation agent (Hurt et al., 1997; Jorenby et al., 1999; Shiffman et al., 2000); however, the mechanism by which bupropion reduces smoking is not fully understood.

Interestingly, acute administration of a low dose of bupropion increased nicotine self-administration, whereas a high dose of bupropion decreased nicotine self-administration in rats, suggesting that bupropion alters nicotine reinforcement (Rauhut et al., 2002). These results are consistent with a recent report that acute bupropion administration increases smoking in non-treatment-seeking smokers (Cousins et al., 2001), while reducing smoking during cessation (Hurt et al., 1997; Jorenby et al., 1999; Shiffman et al., 2000). This biphasic response to bupropion suggests that it has a complex mechanism of action.

The antidepressant effects of bupropion result from inhibition of dopamine and norepinephrine transporters (DAT and NET, respectively); however, its mechanism of action is not fully understood (Ascher et al., 1995). Bupropion inhibits $[^3]H$dopamine ($[^3]H$DA) uptake (IC$_{50}$ = 2 $\mu$M) into rat striatal synaptosomes, $[^3]H$norepinephrine ($[^3]H$NE) uptake (IC$_{50}$ = 5 $\mu$M) into rat hypothalamic synaptosomes, and, less potentially (IC$_{50}$ = 58 $\mu$M), $[^3]H$serotonin uptake into rat hypothalamic synaptosomes (Ferris and Cooper, 1993; Ascher et al., 1995). A competitive interaction with DAT has been demonstrated using $[^3]H$mazindol binding to rat striatal mem-

ABBREVIATIONS: DAT, dopamine transporter; NET, norepinephrine transporter; DA, dopamine; NE, norepinephrine; nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; ANOVA, analysis of variance; *, putative nAChR subtype assignment.
branes (Dersch et al., 1994). Bupropion-induced inhibition of DAT and NET function and associated increases in extracellular DA and NE concentrations, respectively, may substitute for nicotine-evoked neurotransmitter release during smoking, although nicotine reinforcement primarily has been associated with increased DA release (Corrigall et al., 1992). Thus, bupropion inhibition of transporter function likely contributes to its therapeutic efficacy as a smoking cessation agent.

Another mechanism potentially contributing to the efficacy of bupropion as a smoking cessation agent is inhibition of nicotinic acetylcholine receptors (nAChRs). The ability of bupropion to interact with specific nAChR subtypes has been investigated. Bupropion inhibited (IC\textsubscript{50} = 10.5 \mu M) carbamoylcholine (1 mM)-induced \textsuperscript{66}Rb\textsuperscript{+} efflux from human neuroblastoma cells expressing the \(\alpha_3\beta_4\) ganglionic nAChR subtype and more potently inhibited (IC\textsubscript{50} = 1.51 \mu M) \textsuperscript{66}Rb\textsuperscript{+} efflux from human clonal cells expressing the \(\alpha_1\) muscle nAChR subtype (Fryer and Lukas, 1999). Bupropion also inhibited acetylcholine (ACh; 1 \mu M) activation of rat \(\alpha_3\beta_2\) (IC\textsubscript{50} = 1.3 \mu M) and \(\alpha_4\beta_2\) (IC\textsubscript{50} = 8 \mu M) subtypes expressed in Xenopus oocytes (Slemmer et al., 2000). Furthermore, bupropion inhibited the \(\alpha_7\) subtype, but with lower affinity (IC\textsubscript{50} = 60 \mu M). Bupropion-induced inhibition of the above nAChR subtypes was not surmounted by increasing agonist concentrations, indicative of a noncompetitive interaction (Fryer and Lukas, 1999; Slemmer et al., 2000). Interestingly, bupropion (1 and 10 \mu M) did not displace \textsuperscript{3}Hnicotine binding to whole rat brain membranes, also consistent with noncompetitive inhibition of \(\alpha_4\beta_2\) nAChRs (Slemmer et al., 2000). Thus, bupropion noncompetitively inhibits \(\alpha_3\beta_2\), \(\alpha_4\beta_2\), and \(\alpha_3\beta_4\) subtypes when studied using a variety of nAChR expression systems.

Since alterations in both DA and NE neurotransmission likely contribute to the antidepressant effects of bupropion, the present study evaluated the ability of bupropion to inhibit native nAChR subtypes using both \([\textsuperscript{3}H]\)IDA and \([\textsuperscript{3}H]\)NE release assays. Specifically, bupropion inhibition of nicotine-evoked \([\textsuperscript{3}H]\)overflow from superfused rat striatal slices preloaded with \([\textsuperscript{3}H]\)IDA and rat hippocampal slices preloaded with \([\textsuperscript{3}H]\)NE was determined under conditions in which DAT and NET function was inhibited by inclusion of nomifensine and desipramine, respectively, in the superfusion buffer. The exact subunit composition of native nAChRs has not been elucidated conclusively (Lukas et al., 1999). Subtype assignment has been based primarily on the demonstration of inhibition of nicotine response by subtype-selective antagonists in native tissue preparations. However, subtype selectivity of the antagonists has been determined using cell expression systems in which the nAChR subunit composition is known. Nevertheless, converging lines of evidence suggest that nicotine-evoked DA release from striatum and NE release from hippocampus are mediated by \(\alpha_3\beta_2^+\) and \(\alpha_3\beta_4^+\) nAChRs, respectively, although several different nAChR subtypes may be involved in these responses (Kaiser et al., 1998; Luo et al., 1998; Fu et al., 1999; Reuben et al., 2000).

### Materials and Methods

#### Subjects

Male Sprague-Dawley rats (200–250 g) were obtained from Harlan (Indianapolis, IN) and were housed two per cage with free access to food and water in the Division of Laboratory Animal Resources at the College of Pharmacy at the University of Kentucky. Experimental protocols involving the animals were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

#### Chemicals

\((\pm)\)-Bupropion was kindly provided as a gift from Dr. John Reinhard (GlaxoSmithKline, Research Triangle Park, NC). \((S)\)-Nicotine ditartrate was purchased from Sigma/RBI (Natick, MA). Desipramine hydrochloride, mecamylamine hydrochloride, nomifensine maleate, and pargyline hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). \([\textsuperscript{3}H]\)NE (levorotatory) \(N\)-norepinephrine, specific activity 14.4 Ci/mmol and \([\textsuperscript{3}H]\)IDA (3,4-ethyl-2-[\(\textsuperscript{3}H\)]dihydroxyphenylethylamine, specific activity 25.6 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). \(\alpha\)-D-Glucose, \(L\)-ascorbic acid, and TS-2 tissue solubilizer were purchased from Aldrich Chemical (Milwaukee, WI), AnalaR (BHD Ltd., Poole, Dorset, U.K.), and Research Products International (Mount Prospect, IL), respectively. All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

\([\textsuperscript{3}H]\)Overflow Assay. \([\textsuperscript{3}H]\)Overflow from striatal slices preloaded with \([\textsuperscript{3}H]DA\) or \([\textsuperscript{3}H]\)Overflow from hippocampal slices preloaded with \([\textsuperscript{3}H]NE\) was determined using separate groups of rats using modifications of a previously published method (Dwoskin and Zahniser, 1986). Briefly, coronal striatal or hippocampal slices (500 \mu m; 6–8 mg for striatum; 3–4 mg for hippocampus) were incubated in Krebs' buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM Mg\textsubscript{2+}, 1.0 mM Ca\textsubscript{2+}, 13.8 mM NaH\textsubscript{2}PO\textsubscript{4}, 1.3 mM CaCl\textsubscript{2}, 11.1 mM \(\alpha\)-D-glucose, 25.0 mM NaHCO\textsubscript{3}, 0.11 mM L-ascorbic acid, and 4.0 \mu M disodium ethylenediamine tetraacetate, pH 7.4, saturated with 95% O\textsubscript{2}/5% CO\textsubscript{2} in a metabolic shaker at 34°C for 30 min. Slices (six to eight slices/3 ml) were incubated in fresh buffer containing 0.1 \mu M \([\textsuperscript{3}H]DA\) or 0.1 \mu M \([\textsuperscript{3}H]NE\) for an additional 30 min. After rinsing, each individual slice was transferred to a glass superfusion chamber containing two platinum electrodes and maintained at 34°C, and superfused at 1 ml/min with oxygenated Krebs' buffer, containing pargyline (10 \mu M) to ensure that \([\textsuperscript{3}H]\)overflow represented primarily \([\textsuperscript{3}H]IDA\) or \([\textsuperscript{3}H]NE\), rather than their metabolites (Zumstein et al., 1981). In \([\textsuperscript{3}H]IDA\) overflow experiments, nomifensine (10 \mu M) was included in the superfusion buffer to inhibit DAT function. The concentration of nomifensine was based on previous research in which the IC\textsubscript{50} value to inhibit \([\textsuperscript{3}H]IDA\) uptake into rat striatal synaptosomes was ~150 nM (Hunt et al., 1974). In \([\textsuperscript{3}H]NE\) overflow experiments, desipramine (10 \mu M) was included in the superfusion buffer to inhibit NET function. The concentration of desipramine was based on previous research in which the IC\textsubscript{50} value to inhibit \([\textsuperscript{3}H]NE\) uptake into rat hippocampal synaptosomes was 20 nM (Lindbrink et al., 1971; Miller et al., 2002). After 60 min of superfusion, superfusates were collected across the entire sampling period in 5-min fractions (3 ml/sample). Three superfusate samples were collected to determine basal \([\textsuperscript{3}H]\)Outflow. After collection of the third basal sample, slices from an individual rat were superfused for 30 min in the absence or presence of bupropion (1 nM–100 \mu M), and samples were collected to determine intrinsic activity (i.e., ability of bupropion to evoke \([\textsuperscript{3}H]\)overflow). Each slice was exposed to only one concentration of bupropion. Bupropion remained in the buffer throughout the experiment. After 30 min of superfusion in the absence or presence of bupropion, nicotine (10 \mu M) was added to the buffer of each chamber and superfusion continued; samples were collected for an additional 60 min to determine the ability of bupropion to inhibit nicotine-evoked \([\textsuperscript{3}H]\)Overflow. A control slice from each rat was superfused for 30 min with buffer (in the absence of bupropion) followed by superfusion for 60 min with nicotine. The 60-min duration of exposure of the slices to nicotine was chosen based on our previous superfusion experiments determining the effect of nicotine on neurotransmitter release (Dwoskin et al., 1993; Teng et al., 1997), on the observed residence time of nicotine in rat brain (1/2 = 52 min) following a single s.c. injection of nicotine (Crooks et al., 1997; Ghoheh et al., 1998), and on observations from the literature that tobacco smokers maintain a...
Tissue and superfusate samples were processed as previously described. Bupropion using either striatum or hippocampus from a single rat was determined in both the absence and presence of each concentration of bupropion. These experiments utilized a repeated measures design, such that the concentration-response for nicotine was assessed for each subject. Each slice from a single animal was exposed to only one concentration of nicotine and one concentration of bupropion to inhibit [3H]overflow evoked by nicotine (10 nM–100 μM) was determined in the absence or presence of a single concentration of bupropion using brain slices from a single rat. The inhibitory effect of at least three concentrations of bupropion (1–10 μM for [3H]DA experiments or 0.1–10.0 μM for [3H]NE experiments) were determined. Bupropion concentrations were selected based on the respective IC50 values for inhibition of nicotine (10 μM)-evoked [3H]overflow. For [3H]DA or [3H]NE overflow experiments, striatal or hippocampal slices were superfused with buffer containing para-glyine and nomifensine (10 μM) or desipramine (10 μM), respectively, for 75 min. Subsequently, slices were superfused in the absence or presence of nicotine to determine basal [3H]overflow evoked by nicotine (10 nM–10 μM), which remained in the buffer throughout the experiment. After 30 min of superfusion with bupropion, one of six concentrations of nicotine (1 nM–100 μM) was added to the buffer, and superfusion was continued for an additional 60 min. Each slice from a single animal was exposed to only one concentration of nicotine and one concentration of bupropion. These experiments utilized a repeated measures design, such that the concentration-response for nicotine was determined in both the absence and presence of each concentration of bupropion using either striatum or hippocampus from a single rat. The ability of bupropion (10 nM–100 μM) to inhibit [3H]overflow evoked by nicotine (10 μM) to inhibit the [3H]overflow was determined. Each slice was transferred to a glass superfusion chamber maintained at 34°C and was superfused at 1 ml/min with oxygenated Krebs’ buffer containing pargyline (10 μM) and nomifensine (10 μM). After 60 min of superfusion, three 5-min samples (5 ml) were collected to determine basal [3H]overflow. After collection of the third basal sample, striatal slices from an individual rat were superfused for 30 min in the absence or presence of one of several concentrations of mecamylamine (0.01 – 100 μM), which remained in the buffer until the end of the experiment. Each slice was exposed to only one concentration of mecamylamine. Subsequently, bupropion (100 μM) was added to buffer and superfusate samples were collected for an additional 60 min. In each experiment, one slice was superfused in the absence of mecamylamine, and determined the effect of bupropion (100 μM) alone (control condition). For this experiment, mecamylamine concentration was a within-subjects factor.

Data Analysis. Fractional release was calculated by dividing the tritium collected in each sample by the total tritium present in the tissue at the time of sample collection. Fractional release is expressed as a percentage of total tissue tritium (dpm). Basal [3H]overflow was calculated from the average fractional release in the three 5-min samples just prior to the addition of bupropion to the superfusion buffer. Bupropion and nicotine-evoked total [3H]overflow were calculated by summing the increases in fractional release that resulted from exposure to drug and subtracting the basal [3H]overflow across an equivalent period of time.

The intrinsic activity of bupropion on [3H]overflow and the ability of bupropion to inhibit [3H]overflow evoked by 10 μM nicotine were analyzed via one-way repeated measures analysis of variance (ANOVA) with bupropion concentration as a within-subjects factor (SPSS, Version 9.0; SPSS Science, Chicago, IL). Separate analyses were performed for [3H]DA and [3H]NE overflow experiments. Where appropriate, Tukey post hoc tests (p < 0.05) were performed. Time course data were analyzed via two-way repeated measures ANOVA with time and concentration as within-subject factors. EC50 or IC50 values were determined via nonlinear regression to fit the mean data points to a sigmoidal concentration-response curve (Prism, Version 3.0; GraphPad, San Diego, CA).

The mechanism by which bupropion inhibited nicotine-evoked [3H]DA overflow and [3H]NE overflow was determined using separate Schild analyses (Goldstein et al., 1974; Kenakin, 1997). For each experiment using slices from a single rat, the concentration-response for nicotine was determined in both the absence and presence of bupropion, and nonlinear regression was used to fit the concentration-response curves for each experiment, the dose ratio of the equieffective concentration of nicotine in the presence of bupropion to the nicotine concentration in the absence of bupropion was calculated for total [3H]overflow at 0.5% and at 1.0% of tissue tritium content. The log(dose ratio – 1) was plotted as a function of log(bupropion concentration), and linear regression was performed to provide the Schild regression. Additionally, bupropion-induced inhibition of nicotine-induced [3H]overflow was analyzed via three-way repeated measures ANOVA with nicotine concentration and the presence or absence of bupropion as within-subjects factors, and with bupropion concentration as a between-groups factor.

The ability of bupropion to inhibit electrically evoked [3H]overflow was analyzed via two-way repeated measures ANOVA with bupropion concentration as a within-subjects factor and number of electrical pulses as a between-group factor. Mecamylamine-induced inhibition of bupropion (100 μM)-evoked [3H]overflow was analyzed via one-way repeated measures ANOVA with mecamylamine concentration as a within-subjects factor.

Results

Bupropion Competitively Inhibits Nicotine-Evoked [3H]DA Overflow. The ability of bupropion (10 nM–100 μM) to inhibit the electrically evoked [3H]overflow was assessed. Bupropion using either striatum or hippocampus from a single rat was determined in both the absence and presence of each concentration of bupropion using brain slices from a single rat. The inhibitory effect of at least three concentrations of bupropion (1–10 μM for [3H]DA experiments or 0.1–10.0 μM for [3H]NE experiments) were determined. Bupropion concentrations were selected based on the respective IC50 values for inhibition of nicotine (10 μM)-evoked [3H]overflow. For [3H]DA or [3H]NE overflow experiments, striatal or hippocampal slices were superfused with buffer containing para-glyine and nomifensine (10 μM) or desipramine (10 μM), respectively, for 75 min. Subsequently, slices were superfused in the absence or presence of nicotine to determine basal [3H]overflow. Bupropion concentration was a within-subjects factor.

[3H]Overflow. To assess the selectivity of the bupropion-induced inhibition of the effect of nicotine, striatal slices were preloaded with [3H]DA, as previously described, and the ability of mecamylamine (0.01 – 100 μM) to inhibit the [3H]overflow evoked by nicotine (10 μM) was determined. Each slice was transferred to a glass superfusion chamber maintained at 34°C and was superfused at 1 ml/min with oxygenated Krebs’ buffer containing pargyline (10 μM) and nomifensine (10 μM). After 60 min of superfusion, three 5-min samples (5 ml) were collected to determine basal [3H]overflow. After collection of the third basal sample, striatal slices from an individual rat were superfused for 30 min in the absence or presence of one of several concentrations of mecamylamine (0.01 – 100 μM), which remained in the buffer until the end of the experiment. Each slice was exposed to only one concentration of mecamylamine. Subsequently, bupropion (100 μM) was added to buffer and superfusate samples were collected for an additional 60 min. In each experiment, one slice was superfused in the absence of mecamylamine, and determined the effect of bupropion (100 μM) alone (control condition). For this experiment, mecamylamine concentration was a within-subjects factor.

Mecamylamine-Induced Inhibition of Bupropion-Evoked [3H]Overflow. To assess whether bupropion (100 μM)-evoked [3H]overflow (intrinsically) was mediated by nAChRs, striatal slices were preloaded with [3H]DA as previously described, and the ability of mecamylamine (0.01 – 100 μM) to inhibit the [3H]overflow evoked by bupropion was determined. Each slice was transferred to a glass superfusion chamber maintained at 34°C and was superfused at 1 ml/min with oxygenated Krebs’ buffer containing pargyline (10 μM) and nomifensine (10 μM). After 60 min of superfusion, three 5-min samples (5 ml) were collected to determine basal [3H]overflow. After collection of the third basal sample, striatal slices from an individual rat were superfused for 60 min in the absence or presence of a single concentration of bupropion (0.1–10 μM), which remained in the buffer until the end of the experiment. Subsequently, electrical field stimulation was applied, and superfusate samples were collected for an additional 60-min period. For these experiments, the number of pulses was a between-group factor and the bupropion concentration was a within-subjects factor.
to evoke \( ^{3}H \) overflow from superfused rat striatal slices preloaded with \( ^{3}H \) DA was determined (Table 1). A significant main effect of bupropion concentration was found \((F_{5,25} = 5.52, p < 0.001)\). Post hoc tests revealed that 100 \( \mu M \) bupropion significantly increased \( ^{3}H \) overflow above that in the absence of bupropion or during superfusion with lower bupropion concentrations. Thus, only the highest concentration \((100 \mu M)\) of bupropion examined produced intrinsic activity in this assay and, therefore, was not included in the determination of the bupropion-induced inhibition of nicotine-evoked \( ^{3}H \) DA overflow.

In a concentration-dependent manner, bupropion inhibited nicotine \((10 \mu M)\)-evoked \( ^{3}H \) overflow from \( ^{3}H \) DA-preloaded striatal slices, with an \( IC_{50} \) value of 1.27 \( \mu M \) (Fig. 1). A significant main effect of bupropion concentration was found \((F_{5,25} = 9.49, p < 0.001)\). Post hoc tests revealed that 10 \( \mu M \) bupropion inhibited \((-72\%)\) nicotine-evoked \( ^{3}H \) overflow compared with control (i.e., nicotine-evoked \( ^{3}H \) overflow in the absence of bupropion). Analysis of the time course data (Fig. 1, inset) revealed a significant main effect of bupropion concentration \((F_{20,756} = 63.3, p < 0.001)\). A high concentration \((10 \mu M)\) of bupropion significantly decreased fractional release evoked by nicotine across the 60-min period of superfusion with nicotine, relative to control. A lower concentration \((1 \mu M)\) of bupropion significantly inhibited fractional release at the 5 to 35 min of superfusion with nicotine. Thus, at concentrations \((1–10 \mu M)\) that did not intrinsically evoke \( ^{3}H \) overflow, bupropion inhibited nicotine-evoked \( ^{3}H \) overflow from rat striatal slices preloaded with \( ^{3}H \) DA.

To determine the mechanism of the bupropion-induced inhibition of nicotine-evoked \( ^{3}H \) overflow from rat striatal slices preloaded with \( ^{3}H \) DA, a Schild analysis was performed. In each series of experiments, the concentration-response for nicotine \((1 \text{nM}–100 \mu M)\)-evoked \( ^{3}H \) overflow from \( ^{3}H \) DA-preloaded striatal slices was determined in both the absence and presence of one of three bupropion concentrations \((1–10 \mu M)\); Fig. 2). Figure 2 illustrates that increasing concentrations of bupropion shifted the nicotine concentration-response curve parallel and to the right; and at high nicotine concentrations, the bupropion-induced inhibition was surmounted. When the Schild analysis was performed using a \( ^{3}H \) overflow response of 0.5% total tissue tritium, the Schild regression revealed a linear relationship \((F_{1,14} = 16.4, p < 0.01; r^2 = 0.54)\) with a slope of 0.90 (Fig. 2, inset). A linear relationship was also observed for a \( ^{3}H \) overflow response of 1% total tissue tritium \((slope = 0.87; F_{1,15} = 4.52, p = 0.0506; r^2 = 0.23\); regression not shown), indicative of a competitive interaction.

The data from the Schild analysis were also analyzed using ANOVA. A significant main effect of nicotine concentration was found \((F_{6,66} = 15.66, p < 0.001)\), indicating a concentration-dependent increase in nicotine-evoked \( ^{3}H \) overflow across the series of experiments determining inhibition induced by one of the three concentrations of bupropion. The lowest \((1 \mu M)\) concentration of bupropion did not significantly inhibit nicotine-evoked \( ^{3}H \) overflow \((F_{1,5} = 0.51, p = 0.51)\), whereas higher bupropion concentrations \((3 \text{ and } 10 \mu M)\) inhibited nicotine-evoked \( ^{3}H \) overflow \((F_{1,5} = 7.14, p < 0.05\) and \(F_{1,5} = 20.86, p < 0.01\), respectively). Importantly, the inhibition produced by bupropion \((3–10 \mu M)\) was surmounted by superfusion with increasing concentrations of nicotine.

**Bupropion Inhibits Nicotine-Evoked \( ^{3}H \) NE Overflow.** The ability of bupropion \((1 \text{nM}–100 \mu M)\) to evoke \( ^{3}H \) overflow from superfused rat hippocampal slices preloaded with \( ^{3}H \) NE was determined (Table 1). The main effect of bupropion concentration was not significant \((F_{6,30} = 1.38, p = 0.25)\). Thus, across a concentration range of 5 orders of magnitude, bupropion does not stimulate \( ^{3}H \) NE overflow from superfused hippocampal slices.

In a concentration-dependent manner, bupropion inhibited nicotine \((10 \mu M)\)-evoked \( ^{3}H \) overflow from superfused hippocampal slices preloaded with \( ^{3}H \) NE, with an \( IC_{50} \) value of 323 nM (Fig. 3). Analysis of total \( ^{3}H \) overflow following superfusion with bupropion and nicotine revealed a significant main effect of bupropion concentration \((F_{6,416} = 2.12, p < 0.001)\; \text{Fig. 3, inset})\). Bupropion \((1–100 \mu M)\) significantly inhibited nicotine-evoked fractional release at each 5-min time point during the 60-min period of exposure to nicotine compared with control. Bupropion \((0.1 \mu M)\) significantly inhibited \( ^{3}H \) overflow only at the 60-, 75-, and 80-min time points compared with control. Thus, bupropion potently inhibited nicotine-evoked \( ^{3}H \) NE overflow from rat hippocampal slices.

Schild analysis was utilized to determine the mechanism of bupropion-induced inhibition of nicotine-evoked \( ^{3}H \) NE overflow from hippocampal slices by generating concentration-response curves for nicotine \((10 \text{nM}–100 \mu M)\) in the absence and presence of one of four bupropion concentrations \((100 \text{nM}–10 \mu M)\; \text{Fig. 4})\). Figure 4 illustrates that increasing bupropion concentrations \((100 \text{nM}–1 \mu M)\) shifted the nicotine concentration-response curve parallel and to the right; however, the highest bupropion concentration \((10 \mu M)\) shifted the nicotine concentration-response curve to the right and down. Thus, increasing concentrations of nicotine did not surmount the inhibitory effect of the highest bupropion concentration. When a Schild analysis was performed on the data generated from bupropion concentrations of 100 nM to 1

<table>
<thead>
<tr>
<th>Bupropion Concentration</th>
<th>( ^{3}H ) DA Overflow</th>
<th>( ^{3}H ) NE Overflow</th>
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<tbody>
<tr>
<td>0 M</td>
<td>0.01 ± 0.01*</td>
<td>0.043 ± 0.02</td>
</tr>
<tr>
<td>1 nM</td>
<td>ND</td>
<td>0.080 ± 0.042</td>
</tr>
<tr>
<td>10 nM</td>
<td>0.00 ± 0.00</td>
<td>0.020 ± 0.019</td>
</tr>
<tr>
<td>100 nM</td>
<td>0.049 ± 0.041</td>
<td>0.050 ± 0.020</td>
</tr>
<tr>
<td>1 ( \mu M )</td>
<td>0.092 ± 0.077</td>
<td>0.11 ± 0.058</td>
</tr>
<tr>
<td>10 ( \mu M )</td>
<td>0.035 ± 0.028</td>
<td>0.12 ± 0.035</td>
</tr>
<tr>
<td>100 ( \mu M )</td>
<td>0.40 ± 0.16*</td>
<td>0.069 ± 0.033</td>
</tr>
</tbody>
</table>

ND, not determined.
* Data are mean ± S.E.M. of total \( ^{3}H \) overflow expressed as a percentage of tissue tritium.

\( p < 0.05\), compared to 0 to 10 \( \mu M \) bupropion in the \( ^{3}H \) DA series of experiments.

\( n = 6 \) rats/experiment.
of nicotine, a linear relationship (slope = 1.37; Fig. 4, inset) was revealed. A linear relationship was also observed for a 0.5% total tissue tritium (slope = 1.04; F(1,16) = 7.23, p < 0.05; r^2 = 0.31; regression not shown) across the 100 nM to 1 μM concentration range.

Data from this Schild analysis were analyzed also via repeated-measures ANOVA, which revealed a significant three-way interaction of nicotine concentration, the absence or presence of bupropion, and bupropion concentration (F(18,120) = 5.53, p < 0.001). Simple main effect analyses and post hoc tests revealed that nicotine produced a concentration-dependent increase in [3H]overflow across the series of experiments (F(1,20) = 70.3, p < 0.001). The lowest concentration (100 nM) of bupropion examined did not significantly inhibit nicotine-evoked [3H]overflow (data not shown), indicating that these concentrations (0.1–10 μM) of bupropion did not exhibit intrinsic activity. Table 2 provides the results demonstrating that electrical field stimulation-evoked [3H]overflow was not inhibited by bupropion. Electrical field stimulation resulted in total [3H]overflow of 1.6 to 4.7% of [3H]tissue content, which was within the range of [3H]overflow evoked by nicotine in the Schild analysis. ANOVA revealed that neither the main effect of bupropion concentration nor the bupropion concentration x number of pulses interaction was significant (p > 0.05). Thus, bupropion did not inhibit electrical stimulation-evoked [3H]overflow.

Bupropion (100 μM)-Evoked [3H]DA Overflow Is Not Mecamylamine-Sensitive. [3H]DA-preloaded striatal slices were superfused in the absence or presence of
mecamylamine (0.01–100 μM) for 30 min. Subsequently, bupropion (100 μM) was added to the buffer and superfusion continued for 60 min. Prior to the addition of bupropion, mecamylamine did not significantly increase [3H]overflow (p > 0.05, data not shown). Table 3 provides the results demonstrating that bupropion-evoked [3H]overflow was not inhibited by mecamylamine. ANOVA revealed that mecamylamine did not inhibit the bupropion-evoked increase in [3H]overflow (p > 0.05; Table 3). Thus, bupropion-evoked [3H]overflow was not mecamylamine-sensitive.

Discussion

The present study demonstrates that bupropion inhibited nicotine-evoked [3H]DA overflow and [3H]NE overflow from superfused striatal and hippocampal slices, respectively. The interaction of bupropion with DAT or NET was eliminated by inclusion of nomifensine or desipramine, respectively, in the buffer. Thus, bupropion-induced inhibition of the transporters was not involved in the inhibition of nicotine-evoked neurotransmitter release. The highest bupropion concentration (100 μM) evoked [3H]DA overflow but had no intrinsic activity in the [3H]NE release assay. Bupropion concentrations well below those eliciting intrinsic activity inhibited nicotine-evoked [3H]DA and [3H]NE overflow (IC50 values = 1.27 and 0.323 μM, respectively), suggesting antagonist activity at α3β2+ and α3β4+ nAChRs in rat striatum and hippocampus, respectively. Thus, inhibition of these nAChR subtypes was observed across a similar range of concentrations reported to inhibit DAT and NET function (IC50 = 2–5 μM; Ferris and Cooper, 1993).

Although bupropion acted as a nAChR antagonist in several in vitro assays, no intrinsic activity was observed in previous reports. Specifically, bupropion (≥1 mM) did not evoke 86Rb+ efflux from human neuroblastoma cells expressing the α3β4 ganglionic nAChR subtype or from human clonal cells expressing the α1 muscle nAChR subtype (Fryer and Lukas, 1999), nor did bupropion (≥50 μM) elicit current in rat α7, α3β2, or α4β2 subtypes expressed in Xenopus oocytes (Slemmer et al., 2000). Similarly, in the present study, bupropion (1 nM–100 μM) did not evoke [3H]NE overflow from rat hippocampal slices; however, the highest (100 μM) bupropion concentration evoked [3H]DA overflow from striatal slices. Importantly, the bupropion-evoked [3H]DA overflow was not inhibited by mecamylamine, indicating that bupropion-induced intrinsic activity is not mediated by nAChRs. Furthermore, since nomifensine was included in the superfusion buffer in the latter experiments, bupropion-induced intrinsic activity also was not the result of its interaction with DAT.

The observation that bupropion inhibits nicotine-evoked [3H]DA and [3H]NE overflow is consistent with previous findings that other antidepressants, including fluoxetine, desi-
pramine, nisoxetine, citalopram, and nomifensine, inhibited nicotine (100 μM)-evoked [3H]NE overflow from rat hippocampal slices (Hennings et al., 1997, 1999). IC50 values (0.36–1.8 μM) for these antidepressants were similar to those obtained for bupropion in the present study. Studies by Hennings et al. (1997, 1999) did not include an inhibitor of NET in the superfusion buffer throughout the experiment, such that NET likely played a role in the inhibition of nicotine-evoked [3H]NE overflow. Nevertheless, antidepressant-induced inhibition of nicotine-evoked [3H]NE overflow was not correlated with inhibition of NET function, indicating that NET was not involved in the inhibition of NE release (Hennings et al., 1997, 1999). Inclusion of nomifensine or desipramine in the buffer throughout the present experiments was aimed at eliminating DAT or NET function to allow a more direct investigation of the role of nAChRs in bupropion-induced inhibition of nicotine-evoked neurotransmitter release.

To determine whether the inhibitory effect of bupropion on nicotine-evoked [3H]overflow was specific, striatal slices were depolarized by electrical field stimulation, and the effect of bupropion was determined. The field stimulation parameters chosen provided [3H]overflow equivalent to that evoked by superfusion across the range of nicotine concentrations utilized in the Schild analysis. Bupropion did not inhibit [3H]DA overflow evoked by electrical field stimulation. Thus, these results suggest that the bupropion-induced inhibition of the effect of nicotine is mediated by a specific effect at nAChR sites on dopaminergic terminals in striatum. Bupropion interaction with specific nAChR subtypes has been investigated using cell expression systems. Bupropion inhibited the α3β4 ganglionic nAChR subtype expressed in human neuroblastoma cells (Fryer and Lukas, 1999), as well as rat α3β2 and α4β2 expressed in Xenopus oocytes (Slemmer et al., 2000). The current study extends the latter work by demonstrating that bupropion inhibits native nAChRs; however, the exact subunit composition of native nAChRs has not been elucidated conclusively (Lukas et al., 1999).

Subtype assignment of native receptors has been based primarily on inhibition of nicotinic agonist response by subtype-selective antagonists, defined by their inhibitory activity in cell systems expressing nAChR subunits of known composition. Regarding DA release, neuronal bungarotoxin and α-conotoxin-MII selectively inhibit ACh electrophysiological responses in Xenopus oocytes expressing the α3β2 subtype (Luetje et al., 1990; Cartier et al., 1996). These α3β2 subtype-selective antagonists inhibit nicotine-evoked [3H]DA overflow from rodent striatal preparations (Schulz and Zig-
Evidence has accumulated that different nAChR subtypes are responsible for agonist stimulation of DA and NE release. Rank order of potency differed for nAChR agonists to evoke [3H]DA and [3H]NE release from rat striatal and hippocampal synaptosomes, respectively, suggesting involvement of different nAChR subtypes (Reuben et al., 2000). α-Conotoxin AuIB inhibited electrophysiological responses to ACh in Xenopus oocytes expressing α3β2+ nAChRs with 100-fold greater potency than it inhibited responses in oocytes expressing α3β2 or other subunit combinations, indicating α-conotoxin AuIB selectivity for α3β4 (Luo et al., 1998). α-Conotoxin AuIB inhibited nicotine-evoked [3H]NE release from rat hippocampal synaptosomes.
but not nicotine-evoked [3H]DA release from striatal synaptosomes, suggesting that α3β4+ is responsible for nicotine-evoked [3H]NE release (Luo et al., 1998). However, a-conotoxin AuIB only partially inhibited (84%) nicotine-evoked [3H]NE release, suggesting that other subunit combinations may also be implicated. a-Conotoxin MII did not inhibit nicotine-evoked [3H]NE release, indicating that α3β2+ nAChRs are not involved (Luo et al., 1998). Thus, strong evidence was obtained for a role for α3β4+ in agonist-stimulated NE release from hippocampus. However, other investigators have reported that the α3β2-selective antagonists neuronal bungarotoxin and a-conotoxin MII inhibited nicotine-evoked [3H]NE overflow from superfused rat hippocampal slices and nicotine-evoked NE efflux during hippocampal microdialysis, respectively, suggesting that α3β2+ may be involved (Sershen et al., 1997; Fu et al., 1999). Furthermore, α3, α6, β2, and β4 subunit mRNA localization to NE-containing neurons (Wada et al., 1989; Dineley-Miller and Patrick, 1992; LeNovère et al., 1996) suggests their combination with α3β4 to modulate nicotine-evoked hippocampal NE release.

In the present study, bupropion inhibited nicotine-evoked [3H]NE overflow ~4-fold more potently than it inhibited [3H]DA overflow. Since different nAChR subtypes are likely responsible for these responses, the present results indicate that bupropion lacks selectivity in its inhibition of native nAChRs. Furthermore, the present results suggest similar potency for inhibition of α3β2- and α3β4+ subtypes and are in good agreement with studies showing that bupropion inhibited α3β2 and β4 with similar potency when these subunits were expressed in cell systems (Fryer and Lukas, 1999; Slemmer et al., 2000). Although the exact subunit combination for native receptors is unknown, agreement between the present results and those from expressions systems provides evidence that bupropion inhibits these nAChR subtypes.

The present results from Schild analyses revealed that bupropion-induced inhibition of nicotine-evoked [3H]DA overflow from striatal slices was via a competitive interaction with α3β2+ nAChRs. The Schild regression was not significantly different from linearity and had a slope of unity, indicating competitive antagonism at this nAChR subtype. Bupropion-induced inhibition of nicotine-evoked [3H]DA overflow was surmountable with increasing concentrations of nicotine. The competitive nature of bupropion inhibition using native α3β2+ nAChRs in the current study contrasts with the noncompetitive interaction of bupropion at recombinant α3β2 (Slemmer et al., 2000). In the present study, the highest concentration (10 μM) of bupropion nearly completely inhibited the effect of 1 nM to 10 μM nicotine in the [3H]DA release assay, whereas inhibition was completely surmounted by the highest concentration (100 μM) of nicotine examined. However, a caveat of the interpretation that bupropion acts as a competitive α3β2+ nAChR antagonist should be considered based on previous findings. Using either superfused mouse striatal synaptosomes or rat striatal slices, [3H]DA overflow evoked by 100 μM nicotine was only partially inhibited by the classical nAChR antagonist mecamylamine, indicating that the striatal response at this high nicotine concentration is not completely dependent on nAChR activation (Grady et al., 1992; Teng et al., 1997). Therefore, the competitive nature of bupropion interaction with α3β2+ nAChRs is obscured to some extent by non-nicotinic actions of the highest nicotine concentration utilized.

Results from the Schild analysis of bupropion-induced inhibition of nicotine-evoked [3H]NE overflow from rat hippocampal slices are consistent with an interpretation of α3β4+ nAChR reserve. Across low concentrations of bupropion (100 nM–1 μM), inhibition was surmounted with increasing concentrations of nicotine, and the nicotine concentration-response curves appeared shifted in a rightward parallel fashion. Across these bupropion concentrations, Schild regression yielded linearity with a slope of unity, appearing to indicate competitive antagonism. However, inhibition produced by 10 μM bupropion was clearly not surmounted by increasing concentrations of nicotine, even at 100 μM nicotine. These data are consistent with the classic definition of spare receptors (Goldstein et al., 1974). Classically, maximal response is obtained only when a fraction of the total receptor pool is occupied, such that parallel rightward shifts of the curve give the appearance of competitive antagonism; however, when the antagonist has eliminated the receptor reserve, further inhibition of functional receptors decreases the agonist-evoked maximal response because not enough free receptors are available for interaction with agonist. The presence of spare α3β4+ nAChRs in the current assay is consistent with a previous report indicating spare β4-containing nAChRs on adrenal chromaffin cells (Wenger et al., 1997). Furthermore, the present results are consistent with those from previous studies indicating that bupropion acts in a noncompetitive manner to inhibit carbamylcholine-induced 86Rb+ efflux from cells expressing α3β4 ganglionic nAChRs, since this inhibition was not surmounted by increasing carbamylcholine concentrations (Fryer and Lukas, 1999). Thus, the noncompetitive nature of the bupropion interaction with native α3β4+ and recombinant α3β4 nAChRs appears similar; however, receptor reserve appears under native conditions complicates and makes the determination of the mechanism more difficult in hippocampus.

In summary, bupropion-induced inhibition of nicotine-evoked [3H]DA and [3H]NE release by α3β2+ and α3β4+ subtypes, respectively, was observed across a range of bupropion concentrations similar to those that inhibit DAT and NET function. The effect of bupropion to decrease nicotine self-administration may be the result of its inhibition of one or both of these nAChR subtypes. Thus, the combination of bupropion-induced inhibition of native nAChR subtypes and neurotransmitter transporters may provide a beneficial pharmacological profile affording clinical efficacy as both a smoking cessation agent and an antidepressant.

References


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