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European Journal of Pharmacology 474 (2003) 85–93
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Behavioral and biochemical investigations of bupropion metabolites

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Received 20 March 2003; received in revised form 17 June 2003; accepted 24 June 2003

Abstract

The stimulus effects of bupropion metabolites were examined in a drug discrimination procedure using (−)nicotine- and (+)amphetamine-trained rats. (+)- and (−)*threo*hydrobupropion partially substituted in each group. *R*,*R*-hydroxybupropion produced vehicle-appropriate responding in (−)nicotine animals but, when given in combination with the training dose of (−)nicotine, resulted in an attenuated effect. *S*,*S*-Hydroxybupropion partially (66%) substituted for (−)nicotine. In (+)amphetamine-trained animals, *S*,*S*-hydroxybupropion (ED_{50} = 4.4 mg/kg) generalized completely and was similar in potency to bupropion (ED_{50} = 5.4 mg/kg). Bupropion and its metabolites lacked affinity for nicotinic acetylcholinergic receptors, but all antagonized (−)nicotine-induced $^{86}Rb^{+}$ efflux in cells expressing α3β4 nicotinic cholinergic receptors. *S*,*S*-Hydroxybupropion possessed affinity at the dopamine transporter comparable to bupropion, and was also found to bind at the norepinephrine transporter. Although it is unlikely that any metabolite isomer is chiefly responsible for the stimulus actions of bupropion, some probably play a role in the complex actions of this agent.

Keywords: Nicotine; Amphetamine; Bupropion; Bupropion metabolite; Hydroxybupropion; Threohydrobupropion

1. Introduction

Bupropion is an antidepressant medication and an agent used in the treatment of nicotine dependence (i.e., as an adjunct in smoking cessation therapy). Its mechanism of action, in both instances, is unknown. Current thinking is that bupropion might produce some of its effects via inhibition of biogenic amine reuptake; however, it is not particularly potent in this regard (Ascher et al., 1995; Horst and Preskorn, 1998; Sanchez and Hyttel, 1999). Another more recently proposed theory is that the mechanism of action of bupropion, both as an antidepressant and in the treatment of nicotine dependence, might involve nicotinic acetylcholine (nACh or nicotinic) receptors that are linked to dopamine and norepinephrine release (Fryer and Lukas, 1999; Miller et al., 2002; Slemmer et al., 2000). For example, bupropion inhibits nicotine-evoked [^{3}H]dopamine and [^{3}H]norepinephrine overflow from superfused striatal and hippocampal slices, respectively (Miller et al., 2002). In mice, bupropion antagonizes the antinociceptive, motor, hypothermic, and convulsive effects of nicotine (Slemmer et al., 2000). On the other hand, Young and Glennon (2002) and Wiley et al. (2002) have shown that stimulus substitution occurs in dose-dependent fashion when bupropion is administered to rats trained to distinguish (−)nicotine from saline vehicle in a drug discrimination task. Bupropion, then, might owe its therapeutic effectiveness to its ability to antagonize certain actions of nicotine while, at the same time, being able to mimic others. Bupropion itself, however, does not bind at α4β2 nicotinic receptors—the major population of nicotinic acetylcholinergic receptors in the brain—and its stimulus effects, unlike those of nicotine, are not attenuated by the noncompetitive nicotinic cholinergic antagonist mecamylamine (Wiley et al., 2002; Young and Glennon, 2002).

A potentially important question that needs to be addressed is whether any of the above actions are due to specific optical isomers of bupropion or, perhaps, to a bupropion metabolite. Bupropion is a chiral substance, existing both as (+)- and (−)enantiomers; the racemic mixture is employed clinically. Although bupropion has been resolved, the optical isomers rapidly racemize even under neutral conditions (Musso et al., 1993; Fang et al.,

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doi:10.1016/S0014-2999(03)02010-7

2000) suggesting that the observed effects of bupropion in vivo are probably not related to one of its individual enantiomers. Bupropion is rapidly and completely absorbed, and is extensively metabolized in vivo (Rotzinger et al., 1999); less than 10% of a bupropion dose is excreted unchanged (Lai and Schroeder, 1983). Pathways of bupropion metabolism involve hydroxylation of the *tertiary*-butyl group (with or without subsequent cyclization) and/or reduction of the carbonyl group to an alcohol (Schroeder, 1983). There are significant species differences in the metabolism of bupropion (Horst and Preskorn, 1998), but in humans, the two major metabolites are a phenylmorpholinol, hydroxybupropion (BW 306U), and an aminoalcohol, *threo*hydrobupropion (sometimes referred to as *threo*dihydrobupropion, and also known as *R*,*R*-2-(*tert*-butylamino)-1-(3-chlorophenyl)propanol and BW A494U) (Rotzinger et al., 1999; Schroeder, 1983; Suckow et al., 1986; Welch et al., 1987). Another human metabolite, although formed in lesser amounts than the others, is *erythro*hydrobupropion. The same metabolites have been identified in special patient populations such as smokers (Hsyu et al., 1997), alcoholics (DeVane et al., 1990), and the elderly (Sweet et al., 1995). Bupropion metabolites can accumulate in plasma and achieve levels from 10 to 100 times greater than that of the administered agent (Cooper et al., 1984; Suckow et al., 1986). These metabolites would seem to be prime candidates for investigation.

Metabolites of bupropion are not without pharmacologic action. In fact, the pharmacology of certain metabolites has seen some investigation and it has been suggested that the antidepressant actions of bupropion might be due to, or receive contribution(s) from, one or more bupropion metabolites (Martin et al., 1990; Rotzinger et al., 1999; Young, 1991). Evidence suggests that certain bupropion metabolites might contribute to the antidepressant actions of bupropion (Rotzinger et al., 1999; Young, 1991). For example, bupropion is active in several assays indicative of antidepressant action and hydroxybupropion is apparently more ‘‘antidepressant’’ than bupropion (Martin et al., 1990). In locomotor activity tests, bupropion produced dose-related increases in motor behavior in mice but hydroxybupropion and *threo*hydrobupropion, tested at the same doses as bupropion, produced biphasic effects: the lowest doses produced increases in motor counts and the highest dose produced a decrease in activity (Martin et al., 1990). In biochemical assays hydroxybupropion, like bupropion, is a weak inhibitor of monoamine reuptake (Sanchez and Hyttel, 1999), but a fluoro derivative of a hydroxybupropion-related morpholinol is >30 times more potent than bupropion as an inhibitor of norepinephrine reuptake (Kelley et al., 1996).

The purpose of the present investigation was primarily two-fold. First, because bupropion substitutes for nicotine in rats trained to discriminate (−)nicotine from vehicle (Wiley et al., 2002; Young and Glennon, 2002), we synthesized the individual optical isomers of bupropion metabolites (i.e. hydroxybupropion and *threo*hydrobupropion; see Fig. 1 for chemical structures) and examined them in tests of stimulus generalization to determine if they might produce (or be responsible for) the nicotine-like stimulus effects of bupropion. Second, because bupropion possesses some stimulant character, bupropion and isomers of several of its metabolites were also examined in rats trained to discriminate (+)amphetamine from vehicle. Because bupropion is thought to produce some of its effects via nicotinic cholinergic receptors or via transporters, we took advantage of the availability of the metabolite isomers by submitting them to the NIMH Psychoactive Drug Screening Program for examination at several populations of nicotinic receptors and at the dopamine, norepinephrine, and dopamine transporters. *Erythro*hydrobupropion, however, was not examined because it lacks of significant bupropion-like actions (e.g. Martin et al., 1990) and because it is formed to a lesser extent than *threo*hydrobupropion (Posner et al., 1985; Rotzinger et al., 1999; Suckow et al., 1986).

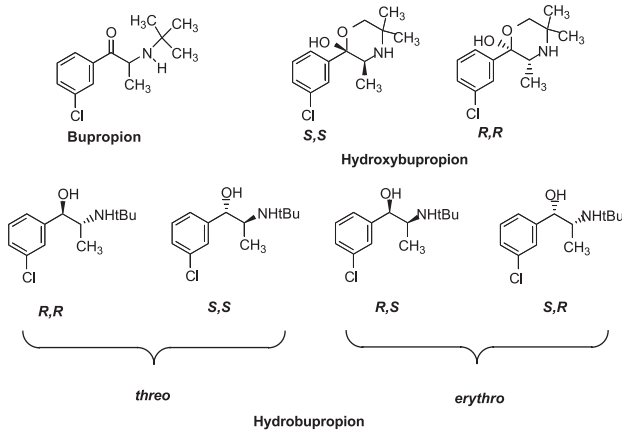

Fig. 1. Chemical structures of bupropion and metabolite isomers *S*,*S*- and *R*,*R*-hydroxybupropion, and *threo*- and *erythro*hydrobupropion.

2. Materials and methods

2.1. Animals

The subjects were 16 male Sprague–Dawley rats (Charles River Laboratories) weighing 250–300 g at the beginning of the study. Nine animals were trained to discriminate 0.6 mg/kg (as the free base) of (−)nicotine, and seven animals were trained to discriminate 1.0 mg/kg of (+)amphetamine sulfate as previously described (Glennon et al., 1995; Young and Glennon, 2002). In brief, the animals were housed individually and, prior to the start of the study, their body weights were reduced to approximately 80% of their free-feeding weight by restricting the availability of food. During the entire course of the study, the animals’ body weights were maintained at this reduced level by restriction of food intake; the animals were allowed drinking water ad lib in their home cages.

2.2. Drug discrimination studies

The animals were trained (15-min training session) to discriminate subcutaneous injections of (−)nicotine or intraperitoneal injections of (+)amphetamine (15-min presession injection interval) from saline vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reward (i.e., sweetened milk) using standard two-lever Coulbourn Instruments operant equipment as previously described (Glennon et al., 1995). Daily training sessions were conducted with the training dose of the training drug or saline. On every fifth day, learning was assessed during an initial 2.5-min non-reinforced (extinction) session followed by a 12.5-min training session. The left lever was designated the drug-appropriate lever for approximately half the animals, whereas the situation was reversed for the remaining animals. Data collected during the extinction session included response rate (i.e., responses per minute) and number of responses on the drug-appropriate lever (expressed as a percent of total responses). Animals were not used in the subsequent stimulus generalization studies until they consistently made >80% of their responses on the drug-appropriate lever after administration of training drug and <20% of their responses on the same drug-appropriate lever after administration of saline.

Tests of stimulus generalization (i.e., substitution) were conducted in order to determine if the training drug stimuli would generalize to bupropion and/or its metabolites. During this phase of the study, maintenance of the training-drug/saline discrimination was insured by continuation of the training sessions on a daily basis (except on a generalization test day; see below). On one of the two days before a generalization test, half the animals would receive the training dose of training drug and the remainder would receive saline; after a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original criteria (i.e., >80% of total responses on the drug-appropriate lever after administration of training drug, and <20% of total responses on the same lever after administration of saline) during the extinction session were excluded from the next generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the training sessions. The animals were allowed 2.5 min to respond under non-reinforcement conditions; the animals were then removed from the operant chambers and returned to their home cages. An odd number of training sessions (usually 5) separated any two generalization test sessions. Doses of test drugs were administered in a random order, using a 15-min presession injection interval, to the groups of rats with the proviso that if a particular dose of drug resulted in behavioral disruption, only lower doses would be investigated in subsequent sessions. Stimulus generalization was considered to have occurred when the animals, after a given dose of drug, made ≥80% of their responses (group mean) on the training drug-appropriate lever. Animals making fewer than five total responses during the 2.5-min extinction session were considered as being disrupted. Response rate data are presented only for animals making ≥5 responses during the extinction session. Where stimulus generalization occurred, ED_{50} values were calculated by the method of Finney (1952). The ED_{50} doses are doses at which the animals would be expected to make 50% of their responses on the drug-appropriate lever.

Tests of stimulus antagonism with *R*,*R*-hydroxybupropion were conducted in a manner similar to the stimulus generalization studies except that the agent was administered 30 min prior to the administration of (−)nicotine (0.6 mg/kg).

2.3. PDSP evaluations

Radioigand binding and $^{86}Rb^{+}$ efflux assays were performed by the NIMH Psychoactive Drug Screening Program (PDSP) using their standard assay protocols.

2.3.1. Radioligand binding assay

Cell lines were established by stably co-transfecting HEK 293 cells with a combination of one rat α nicotinic receptor subunit gene and one rat β subunit gene. Each of the cell lines expressed a single subtype of neuronal nicotinic receptors (including α2β2, α2β4, α3β2, α3β4, α4β2 and α4β4). Binding of [^{3}H](±)epibatidine (PerkinElmer Life Sciences; Boston, MA) to receptors was measured as described previously (Xiao et al., 1998). An initial binding assay, with four replicates, was performed with competition binding experiments using a single concentration of [^{3}H]epibatidine (100 pM), and a single concentration of a test compound (10 μM), and results are expressed as % inhibition of [^{3}H]epibatidine specific binding. More detailed binding assays would be conducted only where >25% inhibition was obtained.

2.3.2. $^{86}Rb^{+}$ efflux assay

Agonist and anagonist activities on the α3β4 receptor subtype were assessed by measuring $^{86}Rb^{+}$ efflux from KXα3β4R2 cells using ^{86}RbCl (2 μCi/ml) (PerkinElmer Life Sciences) as described previously (Xiao et al., 1998). Radioactivity of assay samples and lysates was measured by liquid scintillation counting. Total amount of [^{86}Rb]rubidium chloride ($^{86}Rb^{+}$) loaded (cpm) was calculated as the sum of the assay sample and the lysate of each well. The amount of $^{86}Rb^{+}$ efflux was expressed as a percentage of $^{86}Rb^{+}$ loaded. Stimulated $^{86}Rb^{+}$ efflux was defined as the difference between efflux in presence of nicotinic agonists and basal efflux measured in the absence of agonists. Basal $^{86}Rb^{+}$ efflux ranged from 3% to 6% and maximal stimulated efflux was approximately 45% of loaded $^{86}Rb^{+}$.

A primary assay was conducted with $^{86}Rb^{+}$ efflux experiments. Agonist activity of the test compounds was evaluated at a concentration of 100 μM in the absence of any other

nicotinic agent. Agonist activity is scaled as % of stimulation by 100 μM nicotine. For assessing antagonist activity, three concentrations of a test compound (1, 10 and 100 μM) were applied in the presence of 100 μM nicotine. Antagonist activity is scaled as % inhibition of $^{86}Rb^{+}$ efflux stimulated by 100 μM nicotine. All efflux assays were performed with four replicates.

Because antagonist activity was observed, additional studies were conducted. Eight concentrations of test compound were applied in the absence of nicotine to generate a stimulation curve (for agonist activity) or in the presence of 100 μM nicotine to generate an inhibition curve (for antagonist activity). All efflux assays were performed with four replicates. EC_{50} values for stimulation or IC_{50} values for inhibition were determined by nonlinear least-squares regression analyses (GraphPad, San Diego, CA).

2.4. Drugs

S(−)nicotine hydrogen tartrate and bupropion HCl were purchased from Sigma-Aldrich (St. Louis, MO) and *S*(+)amphetamine sulfate was available in our laboratory from previous investigations. Synthesis of the bupropion metabolites has been previously described and literature methods were employed to obtain the isomers (Musso et al., 1993; Fang et al., 2001). The two *threo* isomers were isolated as their maleate salts whereas the hydroxybupropion isomers were obtained as their hydrochloride salts. The melting points and optical rotations of the individual optical isomers were comparable to reported values.

Doses refer to the weight of the salts, except for nicotine which is reported in terms of the free base. All solutions were prepared fresh daily and subcutaneous (nicotine-trained rats) or intraperitoneal (amphetamine-trained animals) injections were made 15 min prior to testing unless otherwise noted.

Animal studies were conducted under an approved Institutional Animal Care and Use Committee protocol.

3. Results

3.1. Nicotine-trained animals

A group of nine animals was trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle (ED_{50} = 0.11 mg/kg; 95% CL = 0.06–0.20 mg/kg) (Fig. 2). The animals' response rates following administration of saline or the training dose of (−)nicotine were similar (i.e. approximately 20 resp/min). Seven doses of (−)*threo*hydrobupropion (3–21 mg/kg) were examined in the (−)nicotine-trained rats; a dose of 16 mg/kg produced 43% (−)nicotine-appropriate responding (Fig. 2), and administration of higher doses (18 and 21 mg/kg) produced a similar degree of responding. The animals' response rate following 16 mg/kg was comparable to control rates (i.e., rates following administration of either saline or the training dose of (−)nicotine); however, the animals' response rates were depressed following administration of 18 and 21 mg/kg. Six dose of (+)*threo*hydrobupropion were examined. (+)*Threo*-hydrobupropion produced a maximum of 31% (−)nicotine-appropriate responding at 15 mg/kg and saline-appropriate responding at all other doses examined, including a dose (i.e., 21 mg/kg) at which bupropion substituted in (−)nicotine-trained rats (Young and Glennon, 2002).

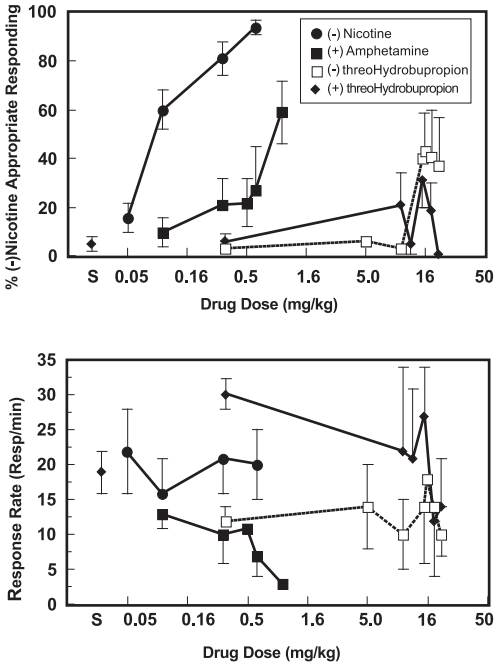

Fig. 2. Results of stimulus generalization studies (upper panel) and animals' response rates (lower panel) using rats (n = 9) trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle. Agents shown are (−)nicotine (solid circles), (+)amphetamine (solid squares), (−)*threo*hydrobupropion (open squares) and (+)*threo*hydrobupropion (solid diamonds); S = 0.9% saline vehicle.

Administration of the major metabolite of bupropion, *R*,*R*-hydroxybupropion, resulted in saline-appropriate responding at all four doses (3–12 mg/kg) examined; at the highest dose evaluated, the animals' response rate was depressed by >50% (Fig. 3). In contrast, *S*,*S*-hydroxybupropion resulted in partial generalization; doses of 11 and 11.5 mg/kg elicited 66% and 55%, respectively, (−)nicotine-appropriate responding. The animals' response rate was depressed by about 50% at the highest dose tested. *R*,*R*-Hydroxybupropion, due to its failure to result even in partial generalization, was examined as a potential antagonist. Administration of doses of this isomer in combination with 0.6 mg/kg of (−)nicotine produced a dose-related partial antagonism of the stimulus, and 20 mg/kg of *R*,*R*-hydroxybupropion in combination with the training dose of (−)nicotine resulted in 54% (−)nicotine-appropriate responding. The animals' response rate at this dose combination was <50% that of control with six of nine animals

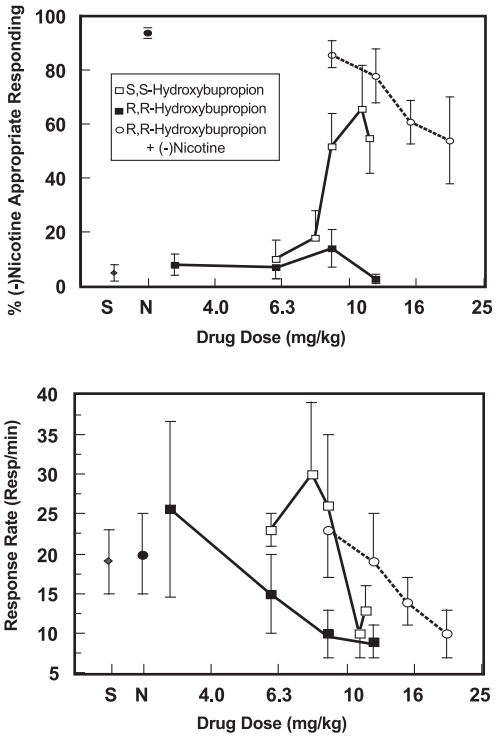

Fig. 3. Results of stimulus generalization studies (upper panel) and animals' response rates (lower panel) using rats ($n=9$) trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle. Agents shown are *S*,*S*-hydroxybupropion (open squares) and *R*,*R*-hydroxybupropion (solid squares). Also shown is the effect of *R*,*R*-hydroxybupropion in combination with 0.6 mg/kg of (−)nicotine (open circles). *S*=0.9% saline vehicle; *N*=0.6 mg/kg of (−)nicotine.

making >2.5 responses/min during the entire extinction session. A limited quantity of this metabolite precluded further testing.

Finally, the effect of six doses of (+)amphetamine was examined (Fig. 2). At 1 mg/kg, (+)amphetamine produced 60% (−)nicotine-appropriate responding; at this dose, only three of six animals responded and their response rate was severely depressed (to about 15% of control). Administration of 1.25 mg/kg of (+)amphetamine disrupted the lever-pressing behavior of all the animals (i.e., none of the animals made ≥5 responses during the entire 2.5-min extinction session).

3.2. (+)Amphetamine-trained animals

Seven rats were trained to discriminate 1 mg/kg of (+)amphetamine ($ED_{50}=0.3$ mg/kg; 95% CL=0.2–0.5 mg/kg) from saline vehicle (Fig. 4). The animals' response rates following administration of saline or the training dose of (+)amphetamine were similar (about 12–15 resp/min). Bupropion ($ED_{50}=5.4$ mg/kg; 95% CL=3.7–7.8 mg/kg) substituted for (+)amphetamine in a dose-related fashion. *S*,*S*-Hydroxybupropion also substituted in a dose-related manner ($ED_{50}=4.4$ mg/kg; 95% CL=1.9–10.4 mg/kg) and was nearly equivalent in potency to bupropion. Four doses of (−)*threo*hydrobupropion (5–21 mg/kg) were examined in these animals (Fig. 5); a dose of 16 mg/kg produced 46% (+)amphetamine-appropriate responding, and administration of 21 mg/kg produced a reduction in response rate. Six doses of (+)*threo*hydrobupropion (5–21 mg/kg) were examined; at 21 mg/kg this compound produced 57% (+)amphetamine-appropriate responding. At this dose, however, only three of five animals responded and their response rate declined to approximately 30% of the control (i.e., (+)amphetamine) response rate.

3.3. Radioligand binding assays

Radioigand binding assays were performed by the NIMH Psychoactive Drug Screening Program. Binding of bupropion and each of the four metabolite isomers was examined at a concentration of 10,000 nM at α2β2, α2β4, α3β2, α3β4, α4β2, and α4β4 nicotinic receptors. At a concentration of 10,000 nM, none of the agents competed strongly (i.e. they produced <50% inhibition) for the binding sites labeled by [^{3}H]epibatidine. These same agents were also examined for their ability to bind at the dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters. All agents failed to show significant affinity at the SERT (Ki>10,000 nM) and only *S*,*S*-hydroxybupropion (Ki=3850 ± 570 nM) possessed measurable affinity at the NET. Only bupropion (Ki=1020 ± 175 nM) and *S*,*S*-hydroxybupropion (Ki=1295 ± 280 nM) displayed affinity for DAT.

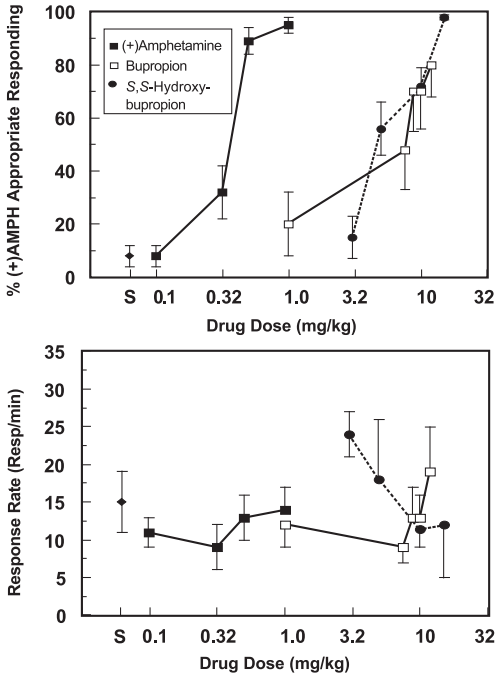

Fig. 4. Results of stimulus generalization studies (upper panel) and animals' response rates (lower panel) using rats ($n=7$) trained to discriminate 1.0 mg/kg of (+)amphetamine from saline vehicle. Agents shown are (+)amphetamine (solid squares), bupropion (open squares), *S*,*S*-hydroxybupropion (solid circles; hatched line); *S*=0.9% saline vehicle.

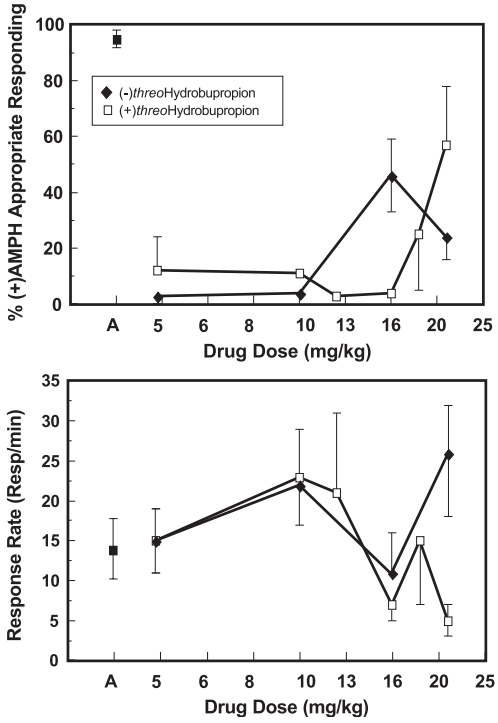

Fig. 5. Results of stimulus generalization studies (upper panel) and animals' response rates (lower panel) using rats ($n = 3-5$) trained to discriminate 1.0 mg/kg of (+)amphetamine from saline vehicle. Agents shown are (−)*threo*hydrobupropion (solid diamonds) and (+)*threo*hydrobupropion (open squares); $A = 1.0$ mg/kg of (+)amphetamine. At a (+)*threo*hydrobupropion dose of 21 mg/kg, only 3 of 5 animals made ≥5 responses during the entire 2.5-min extinction session.

3.4. $^{86}Rb^{+}$ efflux

These studies were also conducted by the NIMH Psychoactive Drug Screening Program. None of the agents examined showed agonist activity as compared with nicotine. Results obtained with bupropion on $^{86}Rb^{+}$ efflux in cells expressing α3β4 receptors showed that it was able to attenuate the effect of 100 μM (−)nicotine by 64% and 96% at concentrations of 10 and 100 μM, respectively. The four metabolite isomers behaved in a similar manner; all isomers were without agonist effect at a concentrations of 100 μM, but inhibited the actions of (−)nicotine by 40% (37–43%) at 10 μM, and by ≥90% at 100 μM. IC_{50} values obtained for bupropion and its metabolite isomers were as follows: bupropion, 7 μM; (−)*threo*hydrobuproion, 14 μM; (+)*threo*hydrobupropion, 14 μM; *R,R*-hydroxybupropion, 20 μM and *S,S*-hydroxybupropion, 18 μM.

4. Discussion

The phenylmorpholinol hydroxybupropion possesses two chiral centers and can exist as two pairs of enantiomers. In theory, all four isomers are possible, but it is believed that the actual metabolite is a mixture of the *S,S*- and *R,R*-isomers (Fang et al., 2001) (see Fig. 1). Most pharmacological studies have simply identified or utilized hydroxybupropion without mention of its stereochemistry. In 1997 Suckow et al. (1997) reported that the actual human metabolite is predominantly the *R,R* isomer. In contrast, although many studies failed to indicate stereochemistry, the predominant aminoalcohol metabolites are believed to be *R,R-threo*hydrobupropion and *R,S-erythro*hydrobupropion (Suckow et al., 1986) (see Fig. 1). With ethanol as solvent, *S,S-threo*dihyrobupropion hydrochloride is (−)*threo*dihydrobupropion (Q. F. Fang, personal communication). The individual isomers (i.e., *S,S* and *R,R*) of hydroxybupropion and *threo*hydobupropion have been described in detail only recently (Fang et al., 2001; Morgan and Partridge, 1999). Interestingly, it is *S,S*-hydroxybupropion—the purportedly minor hydroxybupropion metabolite—that retains the biogenic amine reuptake properties of bupropion, whereas the *R,R*-isomer was less active (Morgan and Partridge, 1999).

4.1. Drug discrimination studies

Recent reports have shown that a (−)nicotine stimulus generalizes to bupropion (Wiley et al., 2002; Young and Glennon, 2002). In humans, bupropion is metabolized primarily to the aminoalcohol *threo*hydrobupropion and the phenylmorpholinol hydroxybupropion. Although (+)*threo*hydrobupropion and *R,R*-hydroxybupropion seem to be the predominant human bupropion metabolites, (+)- and (−)*threo*hydrobupropion, and *R,R*- and *S,S*-hydroxybupropion, were synthesized and examined in the present investigation to determine whether they might contribute to the stimulus actions of their parent. (+)*Threo*hydrobupropion, up to doses of bupropion that substituted for (−)nicotine (i.e., 21 mg/kg) produced a maximum of 31% drug-appropriate responding (Fig. 2). Likewise, (−)*threo*hydrohydrobupropion produced a maximum of 43% drug-appropriate responding (Fig. 2). Hence, although the *threo* isomers might contribute to the overall nicotine-like actions of bupropion, it is highly unlikely that by themselves they are responsible for its stimulus effects.

The major bupropion metabolite, *R,R*-hydroxybupropion, produced saline-appropriate responding at the highest dose examined (Fig. 3). However, when administered in combination with (−)nicotine, *R,R*-hydoxybupropion dose-dependently attenuated the nicotine effect by as much as 50%. An examination of higher doses of this metabolite was precluded due to limited quantities of this agent. These results suggest, if *R,R*-hydroxybupropion is a major metabolite of bupropion in an animal species, it might actually counter an action of nicotine. It should be noted that, following bupropion administration, a relatively low level of hydroxybupropion occurs in the plasma of rat as compared to the relatively high level achieved by this metabolite in the plasma of mouse, dog, and human (Welch et al., 1987). This species difference in metabolism might help

explain why Slemmer et al. (2000) were able to show that bupropion can function as a nicotine antagonist in mice and why Young and Glennon (2002) and Wiley et al. (2002) were unable to demonstrate attenuation of the nicotine stimulus effect by bupropion in rats. Thus, the presence (e.g. in mice) or absence (e.g. in rats) of *R*,*R*-hydroxybupropion could determine the likelihood that an effect would be blocked by bupropion. This conclusion might be important because it has been suggested that bupropion antagonism of the effects of nicotine can contribute to its usefulness in nicotine dependence treatment (Slemmer et al., 2000).

S,*S*-Hydroxybupropion, in contrast to *R*,*R*-hydroxybupropion, partially substituted for the (−)nicotine stimulus (Fig. 3); doses of 11 and 11.5 mg/kg produced between 55% and 66% (−)nicotine-appropriate responding. If *S*,*S*-hydroxybupropion is a significant metabolite in an animal species, it might contribute, along with bupropion itself and the isomers of *threo*hydrobupropion, to the stimulus actions of bupropion.

Taken together, it would seem that none of the examined metabolites of bupropion is responsible for the stimulus actions of bupropion in (−)nicotine-trained animals. That is, none of the bupropion metabolites fully substituted for (−)nicotine. Nevertheless, the partial generalization seen with (+)- and (−)*threo*hydrobupropion, and in particular with *S*,*S*-hydroxybupropion, indicate that these isomers might at least possess the potential to contribute to the actions of bupropion.

Bupropion is a weak central stimulant, and its stimulant properties are similar, yet different, from those of amphetamine. Amphetamine has been consistently shown to elicit only partial generalization in (−)nicotine-trained animals (e.g. Chance et al., 1977; Stolerman et al., 1984). In the present investigation (+)amphetamine administration also resulted in partial generalization, as did (+)*threo*hydrobupropion and *S*,*S*-hydroxybupropion. As already mentioned, *S*,*S*-hydroxybupropion retains the biogenic amine reuptake activity of bupropion (Morgan and Partridge, 1999). As a consequence, it was of interest to determine if this isomer, like bupropion, also retains its amphetamine-like stimulus character. It has been previously demonstrated in tests of stimulus generalization that bupropion substitutes for (+)amphetamine (e.g. Evans and Johanson, 1987), and the structurally related methamphetamine (Munzar and Goldberg, 2000), or produces a high level (77% at 20 mg/kg) of partial generalization (Porsolt et al., 1982). A direct comparison of bupropion and *S*,*S*-hydroxybupropion showed that, indeed, both substitute for (+)amphetamine (Fig. 4). Bupropion (ED_{50} = 5.4 mg/kg) and *S*,*S*-hydroxybupropion (ED_{50} = 4.4 mg/kg) are nearly equipotent, but are about 15 times less potent than (+)amphetamine itself (ED_{50} = 0.3 mg/kg). Numerous doses of the two *threo*hydrobupropion isomers were also examined for purpose of comparison, and both produced partial generalization in the (+)amphetamine-trained animals.

One conclusion of the present investigation is that the nicotine-like stimulus effects of bupropion are associated more with the parent agent than with either (+)*threo*hydrobupropion, (−)*threo*hydrobupropion, *R*,*R*-hydroxybupropion, or *S*,*S*-hydroxybupropion. (−)*Threo*hydrobupropion and *S*,*S*-hydroxybupropion both resulted in partial generalization. However, because these effects were observed at doses higher than the ED_{50} dose of bupropion (ED_{50} = 5.5 mg/kg), it is unlikely that these metabolites, administered acutely, contribute significantly to bupropion's nicotine-like actions. Furthermore, these isomers are not major metabolites of bupropion in humans; thus, it would seem unlikely that they play a primary role in the human pharmacology of bupropion given the doses at which their effects were evident. In contrast, *S*,*S*-hydroxybupropion, like bupropion, substituted for (+)amphetamine, and is even slightly more potent than bupropion in this regard. Here too, however, because *S*,*S*-hydroxybupropion is only a minor bupropion metabolite it seems unlikely that, administered acutely, it contributes significantly to bupropion's effects in (+)amphetamine-trained animals. Nevertheless, because metabolites of bupropion are accumulated in vivo, a role for *S*,*S*-hydroxybupropion cannot be ruled out in the long term effects of bupropion in humans, and this metabolite might account for (or contribute to) some of the weak central stimulant actions of bupropion. Finally, because *R*,*R*-hydroxybupropion—a major metabolite of bupropion—partially antagonized the stimulus effects of (−)nicotine, it would seem that this metabolite could actually detract from the overall "nicotine-like" nature of bupropion. The results presented here indicate that bupropion might produce its stimulus effects via complex mechanisms that could, at least to some extent, involve its metabolites. Bupropion possesses actions of its own; however, certain metabolites mimic or partially mimic these effects, and *R*,*R*-hydrobupropion can at least partially antagonize the actions of nicotine.

4.2. Biochemical studies

Fryer and Lukas (1999) hypothesized that the antidepressant actions of bupropion and its utility in the treatment of nicotine dependence might involve, at least in part, inhibition of nicotinic cholinergic receptor function. Bupropion lacks affinity for the major population of brain nicotinic receptors (i.e., α4β2 receptors) but, nevertheless, acts as a noncompetitive antagonist of acetylcholine-induced currents in frog oocyte preparations expressing α4β2 receptors (Slemmer et al., 2000). Likewise, bupropion behaved as a noncompetitive antagonist of acetylcholine in oocytes expressing α3β2 receptors (Slemmer et al., 2000) and as a noncompetitive antagonist of $^{86}Rb^{+}$-efflux in cell lines expressing α3β4 receptors (Fryer and Lukas, 1999) (but, for further discussion comparing effects on native versus recombinant α3β4 nicotinic receptors, see Miller et al., 2002). In the present study, bupropion was re-examined at α4β2 receptors and, consistent with the previous report

(Slemmer et al., 2000), lacked affinity (Ki>10,000 nM). Likewise, all four bupropion metabolite isomers also lacked affinity (Ki>10,000 nM) for α4β2 receptors. Additionally, *R*,*R*- and *S*,*S*-hydroxybupropion and (+)- and (−)-*threo*hydrobupropion, like bupropion itself, lacked appreciable affinity (i.e., Ki>10,000 nM) for α2β2, α2β4, α3β2, α3β4, α4β2, and α4β4 nicotinic receptors. These same agents were also examined for their affinity at the dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters. None of the metabolites displayed affinity for SERT. Bupropion and *S*,*S*-hydroxybupropion exhibited affinity for DAT, but only *S*,*S*-hydroxybupropion showed affinity for NET.

Evidence suggests that nicotine-evoked DA release from striatum, and NE release from hippocampus, might be mediated via α3β2 and α3β4 nACh receptors, respectively. In the present study, bupropion and metabolite isomers were examined in a $^{86}Rb^{+}$ efflux assay in cells expressing α3β4 receptors. Bupropion was found to act as an antagonist (IC_{50} = 7 μM). The various metabolite isomers exhibited the same action but were about 2- to 3-fold less potent than bupropion: (−)*threo*hydrobuproion (IC_{50} = 14 μM), (+)*threo*hydrobupropion (IC_{50} = 14 μM), *R*,*R*-hydroxybupropion (IC_{50} = 20 μM), and *S*,*S*-hydroxybupropion (IC_{50} = 18 μM). For comparison, under similar experimental conditions, the IC_{50} values of several widely used nicotinic antagonists are, 1.2 μM for mecamylamine, 9.6 μM for d-tubocurarine, 100 μM for dihydro-β-erythroidine, and 208 μM for hexamethonium (Xiao et al., 1998). Miller et al. (2002) have speculated that a combination of bupropion-induced inhibition of native nicotinic receptor subtypes and neurotransporters might account for the clinical efficacy of bupropion as an antidepressant and smoking cessation agent. Acknowledging the fact that results obtained from recombinant receptors might not necessarily be identical with those from native receptors, the present results indicate that metabolites of bupropion are biochemically active and that their effects need to be taken into account when assessing the mechanism of action of bupropion.

4.3. Conclusions

In conclusion, none of the bupropion metabolite isomers examined in the present investigation seemed to fully account for the actions of bupropion in nicotine-trained animals. That is, none resulted in stimulus generalization at doses up to those examined for bupropion. In fact, *R*,*R*-hydroxybupropion was able to attenuate the stimulus effect of (−)nicotine in nicotine-trained animals. In contrast, *S*,*S*-hydroxybupropion partially substituted for (−)nicotine; hence, this metabolite isomer might contribute to the nicotine-like actions of bupropion. With regard to the stimulant character of bupropion, *S*,*S*-hydyxybupropion substituted for (+)amphetamine with a potency comparable to that of bupropion. Thus, it seems entirely possible that this metabolite isomer contributes to (or might even be responsible for) the stimulant nature of bupropion. If the actions of bupropion are mediated, at least in part, by its ability to antagonize α3β4 nicotinic acetylcholinergic receptors, this action is shared by all of the metabolites examined; if interaction at the dopamine transporter is important, *S*,*S*-hydroxybupropion binds with an affinity comparable to that of bupropion. It might be recalled, however, that *R*,*R*-rather than *S*,*S*-hydroxybupropion has been reported to be the major morpholinol metabolite of bupropion in humans (Suckow et al., 1997). If this is the case, then the morpholinol metabolite could conceivably interfere with some of the nicotine-like actions of bupropion. It must also be emphasized that the present investigations focused on acute administration of bupropion and its metabolites, and it has been reported that bupropion metabolites are accumulated in plasma following chronic bupropion administration (Cooper et al., 1984; Suckow et al., 1986). Additional studies will be required to ascertain the potential impact of accumulated metabolites in the actions of bupropion.

Acknowledgements

This work was supported in part by DA 01642 and DA 05274. We also wish to acknowledge the NIMH Psychoactive Drug Screening Program for providing the biochemical results on the bupropion metabolite isomers.

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