Evidence that the Acute Behavioral and Electrophysiological Effects of Bupropion (Wellbutrin®) Are Mediated by a Noradrenergic Mechanism

Barrett R. Cooper, Ph.D., Ching M. Wang, Ph.D., Richard F. Cox, Ph.D., Ronald Norton, B.S., Virginia Shea, Ph.D., and Robert M. Ferris, Ph.D.

Bupropion (BW 323U66) has been considered a dopaminergic antidepressant based on its ability to inhibit the uptake of dopamine (DA) somewhat more selectively than it inhibits uptake of norepinephrine (NE) or serotonin (5-HT). This report describes new evidence that bupropion selectively inhibits firing rates of NE cells in the locus coeruleus (LC) at doses significantly lower than those that inhibit activity of midbrain DA cells or dorsal raphe 5-HT cells. The IC_{50} dose (13 mg/kg IP) for inhibition of LC firing produced plasma concentrations that were not significantly different from those generated by the ED_{50} in the Porsolt test (10 mg/kg IP). The fourfold higher dose needed to inhibit DA cell firing (IC_{50} = 42 mg/kg IP) was similar to the dose associated with locomotor stimulation in freely moving rats. Bupropion did not change the firing rates of 5-HT cells in the dorsal raphe nucleus at any dose. In both in vitro and in vivo tests, the metabolite 306U73 (hydroxybupropion), a weak inhibitor of NE uptake, was approximately equipotent to bupropion with regard to inhibition of LC cells. Another metabolite, 494U73, had no effect on LC firing rates over a wide range of doses. Because of species variation in metabolism, 306U73 was not detected in plasma of rats after IV doses of bupropion that inhibited LC firing. Only trace amounts of 306U73 were detected after bupropion dosing for the Porsolt test. Pretreatment with reserpine markedly depleted catecholamines and reduced (by 30-fold) the potency of bupropion to inhibit LC firing. The effects of clonidine, a direct acting α_2 agonist, were not significantly changed by reserpine. Likewise, a reduction in the effect of bupropion on LC firing was observed in vitro after depletion of catecholamines with reserpine or tetrabenazine. These results suggest that bupropion preferentially affects NE neurons in locus coeruleus at doses that are active in animal antidepressant tests. The doses of bupropion required to inhibit DA cell firing were associated with inhibition of DA uptake and behavioral stimulation and were significantly higher than those selectively producing behavioral effects in animal antidepressant tests. The acute electrophysiological actions of bupropion on NE cells require a reserpine-sensitive store of NE and occur at doses having activity in antidepressant screening tests.

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All from the Division of Pharmacology Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709.

Address correspondence to: Barrett R. Cooper, Ph.D., Division of Pharmacology, Burroughs Wellcome Co., 3030 Cornwallis Road, Research Triangle Park, NC 27709.

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Bupropion is a novel antidepressant with an undefined mechanism of action. Studies have shown that it weakly inhibits the uptake of dopamine in vitro (Ferris et al.

1981, 1983). In in vivo experiments with rats, relatively high doses of bupropion (12.5 to 100 mg/kg, IP) were required to antagonize the dopamine-depleting effects of the neurotoxins 6-hydroxydopamine (6-OHDA) (Cooper et al. 1980) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), (Ricuarte et al. 1985; Finnegan et al. 1987). On the other hand, 100 mg/kg of bupropion only minimally attenuates depletion of norepinephrine by 6-OHDA in rat brain (Cooper et al. 1980). This finding is consistent with reports indicating that bupropion is a relatively weak inhibitor of norepinephrine uptake (Ferris et al. 1981; Perumal et al. 1985). Bupropion does not inhibit monoamine oxidase; does not block serotonin uptake; and binds only weakly to serotonergic, monoaminergic, or other central receptor sites in animals (Soroko et al. 1977; Ferris et al. 1981, 1983; Wander et al. 1986) and man (Richelson and Nelson 1984; Richelson and Pfenning 1984) that are associated with the actions of antidepressant drugs. The down-regulation of beta adrenergic receptors by bupropion remains controversial (Sethy and Harris 1982; Ferris and Beaman 1983; Gandolfi et al. 1983).

In animal behavioral studies, bupropion prevents tetrabenazine-induced sedation in mice and stress-induced immobility in rats, actions shared with tricyclic antidepressants that inhibit norepinephrine uptake (Porsolt et al. 1979; Soroko and Maxwell 1983). The objective of this study was to examine the acute effects of bupropion and its major metabolites on the firing rates of monoaminergic neurons in rat brain. The results were then correlated with plasma levels and behavioral actions of the drugs.

METHODS

Animals

Female Sprague-Dawley rats weighing 75 to 100 g (CD strain, Charles River Labs, Raleigh, NC) were used for *in vitro* electrophysiological experiments. Male CD rats weighing 220 to 350 g (Charles River) were used for *in vivo* experiments. The rats were housed in the light- and temperature-controlled animal facility at Burroughs Wellcome Co. Standard rat chow and water were continuously available. At least 5 days were allowed for acclimatization to the animal facility before experiments were conducted.

Drugs and Drug Treatments

Bupropion HCl (BW 323U66), hydroxybupropion (BW 306U73), and threohydrobupropion (BW 494U73) were synthesized at Wellcome Research Laboratories, Research Triangle Park, NC. Yohimbine HCl (Sigma), lysergic acid diethylamide (LSD, Research Triangle Institute), clonidine (Ciba-Geigy), N,N-dimethyltryptamine (Sigma), (±)8-hydroxydipropyl-aminotetralin (8-OH-DPAT, Research Biochemicals, Natick, MA), and piperoxane HCl (Sigma) were obtained from the indicated sources. Compounds were dissolved in isotonic saline for injection by the IV or IP route. For in vitro electrophysiological studies, the compounds were dissolved in artificial cerebral spinal fluid (ACSF). In some studies, tetrabenazine (Hoffman-La Roche) was dissolved in ACSF to 10 μmol/L and superfused over brain slices for 60 minutes and during drug applications. Reserpine (Serpasil, Eli Lilly), 2.5 mg/kg SC, was injected approximately 24 hours before electrophysiological studies or assays of biogenic amines.

In Vitro Single Unit Recording

Brain slices containing the locus coeruleus were prepared by a modification of the methods of Rigdon and Wang (1991). Briefly, rats were decapitated, and their brains were rapidly removed and placed into cool ACSF. Coronal blocks of brain were cut at the level of the cerebellum and attached to a vibratome chuck with cyanoacrylate glue (Duro Quick Gel). Coronal sections (300 μ) were cut on a vibratome (Lancer 1000) submerged in continuously gassed (95% O_2/5% CO_2) ACSF chilled to 4°C. Slices containing locus coeruleus were transferred to a recording chamber and incubated for 60 minutes in ACSF of the following composition (in mM): NaCl 126, KCl 5, NaH_2PO_4 1.2, $CaCl_2.2\ H_2O$ 2.5, $MgCl_2$ 1.3, $NaHCO_3$ 25, and glucose 11. Sections were placed upon a nylon mesh and held in place with surgical gauze weighted with platinum wires. The chamber was superfused at a rate of 2.5 to 3.5 ml/min with ACSF (35°C) that was bubbled with 95% O_2/5% CO_2. Fine tungsten electrodes (3 to 6 mmol/LΩ impedance) were guided into the tissue under a dissecting microscope and used to detect unitary neuronal activity. Action potentials were amplified, filtered with a window discriminator (Fintronics WDR-420), and displayed on an oscilloscope screen. Counts were accumulated over 10-second intervals, and rate histograms were plotted for subsequent analysis. After recording 5 minutes of stable baseline activity, the flow was switched to ACSF containing test compounds until steady-state effects occurred. Data were analyzed by calculating mean firing rate during the last five minutes of each drug application period and expressing it as a percentage of the baseline firing rate. IC_{50} values were calculated from plots of average responses versus the log concentrations of compounds.

In Vivo Single Unit Recording

Standard extracellular recording methods were used to record responses of single neurons in vivo. Rats were anesthetized with chloral hydrate (400 mg/kg IP) and

mounted in a stereotaxic apparatus. The skull was exposed and a small burr hole was drilled over the area of interest. A lateral tail vein was cannulated for IV drug injections and supplemental doses of anesthetic that were given as needed. Body temperature was maintained between 36° and 38°C with a heating pad. Single barrel glass microelectrodes were filled with 2 mmol/L NaCl containing 1% Fast Green dye (3 to 12 MΩ impedance) and vertically introduced through the hole into the areas of the locus coeruleus, dopamine cell groups A9 and A10, or the dorsal raphe nucleus. Action potentials were amplified and simultaneously monitored on an oscilloscope screen and an audiomonitor. Unitary extracellular spikes were detected by a window discriminator, counted during consecutive 10-second intervals, integrated, and plotted as rate histograms.

For recordings of locus coeruleus, electrodes were introduced to 1.0 to 1.2 mm posterior to lambda, 1.0 to 1.2 mm lateral of midline, and lowered to 5.5 to 6.5 mm below the dura. The relevant characteristics of locus coeruleus neurons are as follows: firing rates of 0.5 to 5.0 spikes/sec and response to contralateral foot pinch with brief excitation followed by a period of quiescence (Graham and Aghajanian 1971; Marwah and Aghajanian 1987; Pitts and Marwah 1982). These neurons were situated medial to cells of the mesencephalic nucleus of the trigeminal and just below the fourth ventricle.

Dopaminergic neurons were characterized by long duration (>2.5 msec) biphasic or triphasic waveforms, a slow regular firing pattern or bursting activity with decreasing spike amplitude waveforms, and a low-pitched sound on the audio monitor (White and Wang 1984; Cox et al. 1988; Bunney et al. 1973). Dopamine cells recorded in these experiments had firing rates of 2 to 9 spikes per second. Stereotaxic coordinates for A10 cells were 2.9 to 3.4 mm anterior to lambda, 0.5 to 0.9 mm lateral of midline, and approximately 6.5 to 8.3 mm below dura. Coordinates for A9 cells were 2.9 to 3.4 mm anterior to lambda, 1.5 to 2.2 mm lateral to midline, and 6.5 to 8.3 mm below dura.

Serotonergic cells in the dorsal raphe nucleus were identified by their long duration (1 to 2 msec), positive-negative action potentials, slow (0.2 to 2.5 spikes/sec) firing rates, and very regular firing patterns (Aghajanian and Haigler 1974). Stereotaxic coordinates for the dorsal raphe were 0.3 to 0.7 mm posterior to lambda, 0.0 to 0.2 lateral to midline, and 4.5 to 5.5 mm ventral to the surface of the brain. After experiments with test compounds, 5-HT neurons were tested for response to N,N-dimethyltryptamine, 8-OH-DPAT, or LSD.

In all recordings, spontaneous baseline firing rates were established for 3 to 5 minutes before drug testing. For cumulative dose-response studies by the intravenous route, drugs were injected at 2- to 3-minute intervals in cumulative doses. Responses to drug doses were defined as the average activity during the final minute of each dose interval and were expressed as a percentage of baseline rate. For experiments by the intraperitoneal route, responses were determined as the average firing rate during the final 5 minutes of the 15-minute period following drug injections. Only one cell was studied in each rat by either the IV or IP route. After each recording, −25 μA of current was passed through the recording electrode for 15 minutes to eject dye. Rats were perfused transcardially with phosphate buffered 10% formalin, and their brains were removed and fixed in formalin. Recording sites were verified histologically by visualization of a dye mark in 50-μ frozen sections.

Behavioral Experiments

The antidepressant effects of bupropion were determined with the Porsolt test (Porsolt et al. 1978; Soroko and Maxwell 1983). Rats were given a 15-minute conditioning trial (water temperature 25°C) followed by a test trial 24 hours later. Intraperitoneal injections of bupropion were given immediately after the conditioning trial, then at 5 hours and 1 hour before the 5-minute test trial. After the test trial, animals were returned to their home cages.

Other animals were tested for locomotor activity that was measured in open field activity cages using established methods (Cooper et al. 1980; Soroko and Maxwell 1983).

Analysis of Bupropion Levels and Major Metabolites

Plasma concentrations of bupropion and 306U73 were determined by HPLC analysis with the method of Cooper et al. (1984) as modified by DeVane et al. (1986). Several bupropion dosing protocols were used to obtain plasma levels corresponding to electrophysiological and Porsolt test parameters. For correspondence to the electrophysiological experiments, blood was drawn 5 minutes after bolus IV doses in chloral hydrate anesthetized rats. In other rats, blood was drawn 1 hour after the last of the three injections in the Porsolt test. Concentrations of 306U73 were determined in plasma 5 minutes after IV injections of 306U73.

Determination of Whole Brain Catecholamine Levels

Whole brain catecholamine concentrations were determined in rats sacrificed 24 hours after injection of reserpine (2.5 mg/kg SC). Concentrations of NE, DA, and 5-HT were determined by the HPLC method described in Jones-Humble et al. (1992). Briefly, brains were rapidly removed from reserpine-treated and control rats,

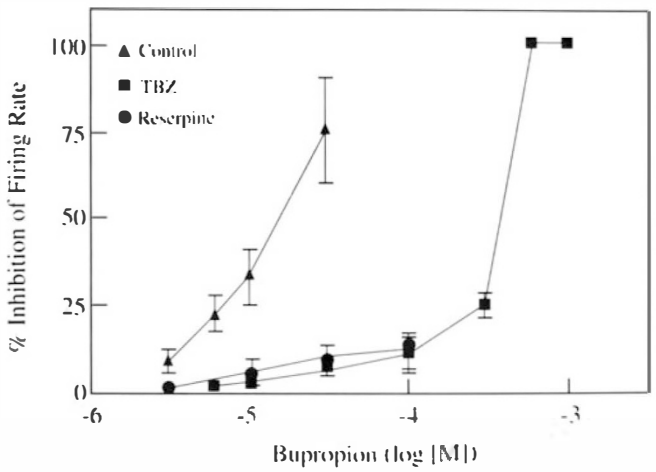

Figure 1. Effects of bupropion on firing rates of locus coeruleus neurons recorded from rat brain slices in vitro. Tissue from untreated control (▲) or reserpine-treated (●) rats, or brain slices exposed to tetrabenazine (■), as described in Methods, were exposed to various concentrations of bupropion (3 to 1000 μmol/L). Each point represents the mean ± standard error of the mean (SEM) of 3 to 6 responses for each concentration of bupropion except at 600 and 1000 μmol/L where $n = 2$.

weighed, and frozen on dry ice. After homogenization and extraction of monoamines, analysis was performed by HPLC with electrochemical detection.

RESULTS

Single-Unit Recording: Locus Coeruleus In Vitro

Firing rates of locus coeruleus neurons in brain slices from control rats were inhibited by an average of 76% with concentrations of bupropion up to 30 μmol/L (Fig. 1). The IC_{50} for this effect was 14.5 ± 0.9 μmol/L ($n = 6$). The metabolite 306U73 inhibited locus coeruleus firing by 85% at 100 μmol/L and was slightly more potent, with an IC_{50} of 9.5 ± 0.4 μmol/L ($n = 6$). Another metabolite, 494U73, did not affect locus coeruleus firing rates at concentrations up to 100 μmol/L.

Depletion of norepinephrine by tetrabenazine in vitro or by reserpine in vivo had pronounced effects on the ability of bupropion to inhibit locus coeruleus neuronal firing (Fig. 1). In brain slices treated with tetrabenazine, bupropion inhibited firing rate, with an IC_{50} that exceeded 300 μmol/L ($n = 6$). In brain slices from reserpinized rats, bupropion inhibited firing by only 11%, with an extrapolated IC_{50} exceeding 8500 μmol/L ($n = 4$). The potency of clonidine in brain slices was not different from control and reserpine-treated rats (IC_{50} = 3 nmol/L, n = 3 to 4 cells/group), and 100% inhibition of firing rate was achieved at 10 nmol/L.

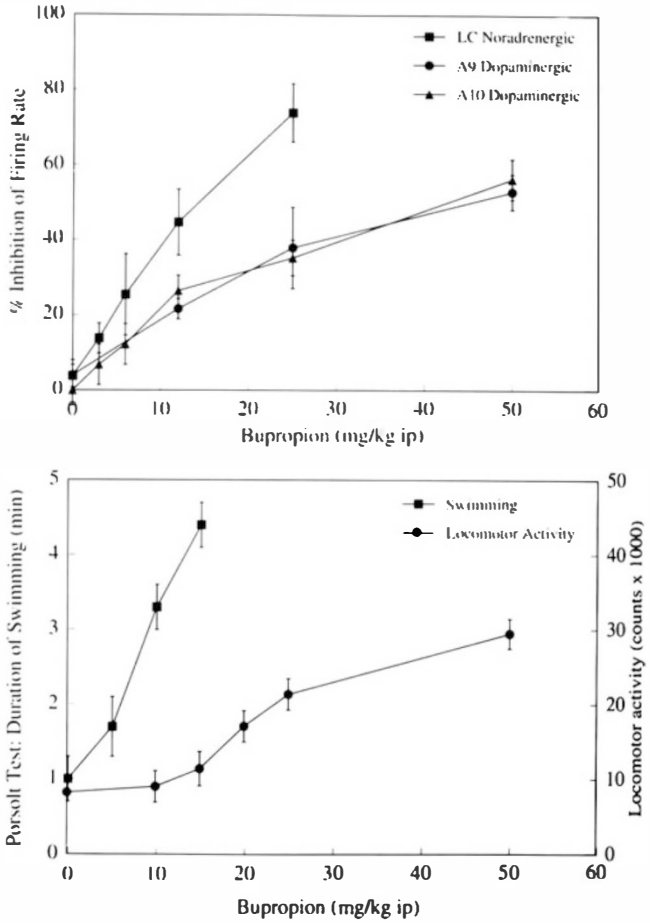

Figure 2. *Top:* Effects of intraperitoneal bupropion on firing rates of neurons from locus coeruleus (■, LC), substantia nigra (●, A9) and ventral tegmental area (▲, A10) in chloral hydrate anesthetized rats. Each point represents the mean ± SEM of 5 to 8 cells at each dose. *Bottom:* Effects of bupropion on duration of swimming in the Porsolt test (■) and on locomotor activity (●) in rats. Swim time was increased by relatively low doses of bupropion with and ED_{50} of 7 mg/kg IP. Locomotor activity was increased only at higher doses (20 to 50 mg/kg IP) iwth an ED_{50} of 17 mg/kg IP.

Single-Unit Recording: Locus Coeruleus In Vivo

In control rats, locus coeruleus firing rates were inhibited by doses of bupropion ranging from 3 to 25 mg/kg IP (Fig. 2, top). Maximum inhibition of firing after 25 mg/kg IP was 74 ± 8% (n = 5 to 8 cells/dose) with an ID_{50} of 12.6 mg/mg IP.

The effects of IV bupropion and its metabolites on locus coeruleus neuronal firing rates are shown in Figure 3. In these animals, bupropion inhibited locus coeruleus firing rate by up to 90%, with an ID_{50} of 2.7 mg/kg IV. Like bupropion, 306U73 also inhibited LC firing, with an ID_{50} of 1.4 mg/kg IV. The metabolite 494U73 had only minor effects on LC firing after doses as high as 12.5 mg/kg IV.

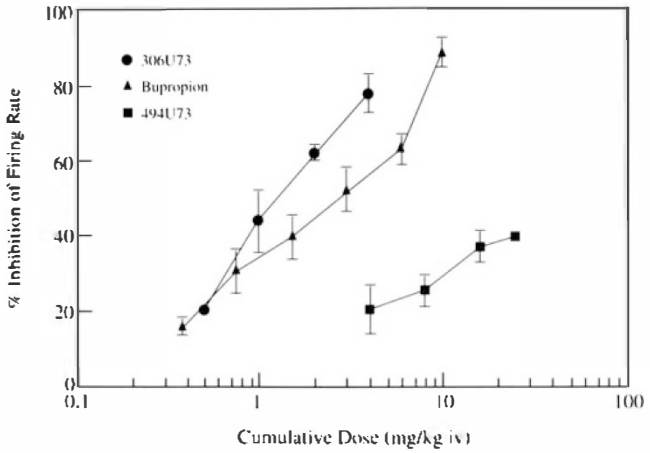

Figure 3. Responses of locus coeruleus neurons to cumulative IV doses of 306U73 (●), bupropion (▲), and 494U73 (■). The ID_{50} values of 306U73 and bupropion are 1.4 and 2.7 mg/kg IV, respectively. Each point represents the mean ± SEM of 3 to 8 cells at each cumulative dose.

In another series of experiments, the effects of bupropion on LC firing rates were examined in control rats and in rats given reserpine 2.5 mg/kg SC 24 hours before recordings. Normal rats (n = 8) had baseline spontaneous firing rates of 29.6 ± 3.4 spikes every 10 seconds. The ID_{50} for bupropion was 1.7 ± 0.5 mg/kg IV, and a maximal effect of 74% inhibition occurred after 6 to 12 mg/kg IV (Figure 4, top). No adverse effects were observed in any control animals.

For reserpinized rats (n = 6), baseline spontaneous firing rates of locus coeruleus neurons (34.2 ± 3.0 spikes/10 sec) were not significantly different from those of control animals. The depletion of monoamines with reserpine resulted in a significant decrease in the potency of bupropion (Fig. 4, top). The ID_{50} was increased by a factor of 30, to 50.8 ± 11.5 mg/kg IV (p = 0.001, two-tailed t-test), and inhibitions of firing rate comparable to those seen in controls (72%) required doses of 100 mg/kg IV. The responses of locus coeruleus neurons to the direct-acting adrenergic agonist clonidine were not affected by pretreatment with reserpine (Figure 4, bottom). Inhibitions of LC firing by bupropion and clonidine in controls or reserpinized rats were reversed by either yohimbine (0.5 mg/kg IV) or piperoxane (0.5 mg/kg IV).

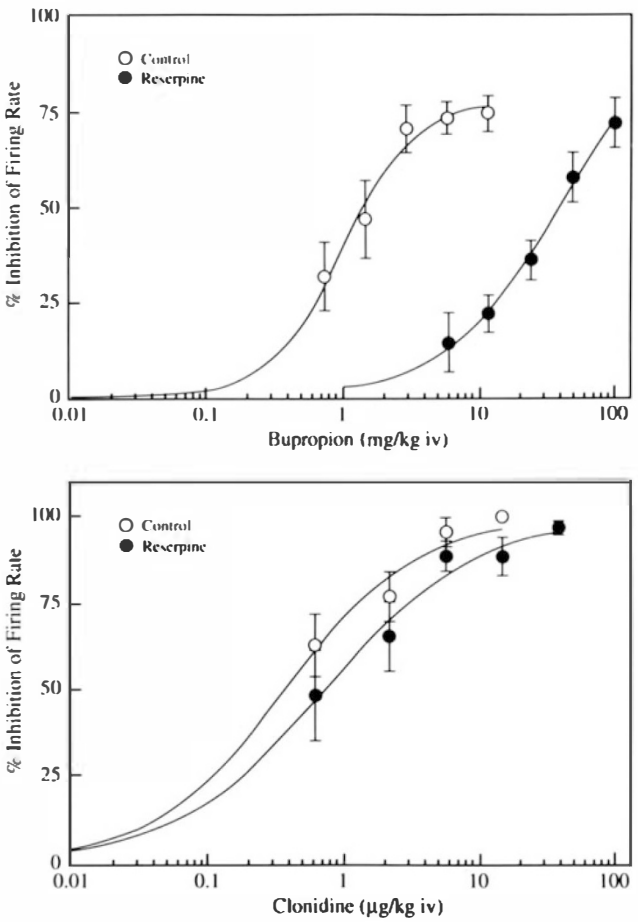

Figure 4. *Top:* Depletion of catecholamines with reserpine (2.5 mg/kg SC, 24 hours) shifted the bupropion dose-response curve rightward with 30-fold less potency compared to untreated controls. Each point represents the mean ± SEM of 2 to 8 cells at the indicated cumulative dose. *Bottom:* Reserpine treatments did not significantly affect the response of locus coeruleus neurons clonidine, a direct acting adrenergic agonist. Each point represents the mean ± SEM response of 3 to 4 cells at each cumulative dose.

Single-Unit Recording: Substantia Nigra (A9) and Ventral Tegmental Area (A10) In Vivo

The firing rates of dopamine neurons in the substantia nigra (A9) and ventral tegmental area (A10) midbrain regions were inhibited in a dose-dependent manner by IV or IP injections of bupropion (Figures 2 and 5). The magnitude of these inhibitions was more limited, and higher doses were required compared to those in locus coeruleus. Intraperitoneal injections of bupropion up to 50 mg/kg produced nearly identical effects on A9 and A10 dopamine cells: 54% (n = 4) and 56% (n = 4) inhibition, respectively. The ID_{50} values for the effects of intraperitoneal bupropion were 43.8 mg/kg for A9 cells and 42.5 mg/kg for A10 cells.

Cumulative intravenous doses of bupropion up to 12 mg/kg inhibited the firing of A9 dopamine neurons by 50% (ID_{50} = 12 mg/kg) and A10 dopamine neurons by 45% (ID_{50} > 12 mg/kg). Inhibitions of dopamine cell firing by bupropion were reversed by the dopamine antagonist haloperidol (50 to 200 µg/kg IV).

494U73 had no effect on A9 cell firing in doses up to 25 mg/kg IV (Figure 5). 306U73, in doses up to 12 mg/kg IV, tended to increase the firing rates of A9 dopamine neurons, but this effect was not statistically significant (p > 0.05, two-tailed t-test).

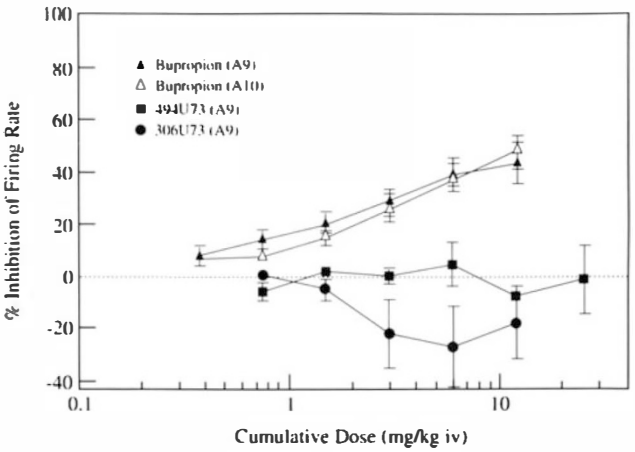

Figure 5. Effects of intravenous bupropion (▲) and its metabolites, 306U73 (●) and 494U73 (■), on the firing rates of A9 (substantia nigra, closed symbols) and A10 (ventral tegmental area, open symbols) dopamine neurons in chloral hydrate anesthetized rats. Each point represents the mean ± SEM of 3 to 6 cells at each cumulative dose.

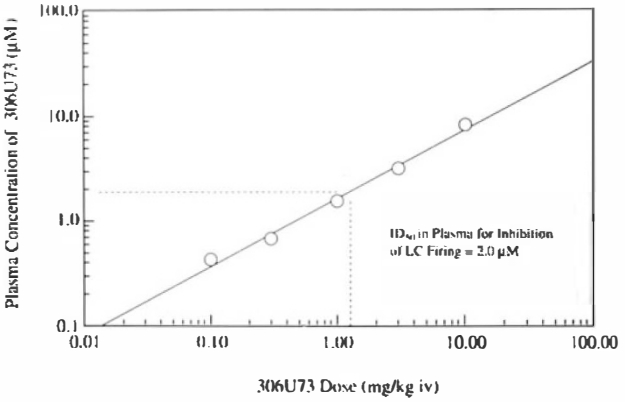

Figure 6. Relationship between IV doses and plasma levels of 306U73 determined 5 minutes after IV doses of 306U73 in rats. The plasma level of 306U73 determined after the IV ID_{50} dose of 306U73 for inhibition of locus coeruleus firing rate was 2.0 μmol/L. No bupropion was detected in rat plasma after doses of 306U73.

Single-Unit Recording: Dorsal Raphe Nucleus In Vivo

Bupropion had no significant effect on the spontaneous firing rates of serotonergic neurons in dorsal raphe nucleus when injected in doses up to 12 mg/kg IV or 25 mg/kg IP. Likewise, 306U73 did not consistently affect 5-HT cell firing after 12 mg/kg IV. There was a weak but highly variable effect of 494U73 on dorsal raphe firing. In contrast, the firing rates of 5-HT neurons unresponsive to these compounds were readily suppressed by injections of N,N-dimethyltryptamine, LSD, or 8-OH-DPAT.

Behavioral Experiments

Figure 2 (bottom) shows the effects of bupropion on swimming activity (Porsolt test) and on locomotor behavior in rats. Bupropion caused a dose-related antagonism of immobility, with an ED_{50} of 7 mg/kg IP.

Table 1. Plasma Concentrations of Bupropion and Its Metabolite 306U73 Following Half Maximal Effective Doses from the Porsolt Test and for Inhibition of Locus Coeruleus Firing

	Plasma Concentration at Time of Test	
Bupropion Dose	**Bupropion**	**306U73**
10 mg/kg IP (Porsolt test)	3.0 μmol/L	0.4 μmol/L
2.7 mg/kg IV (single unit studies)	2.3 μmol/L	ND

ND = not detectable.

In addition, a dose-related stimulation of locomotor activity was seen after higher doses of bupropion (10 to 50 mg/kg IP).

Plasma Levels of Bupropion and Its Metabolites in Various Dosing Schedules

Table 1 shows plasma levels of bupropion in rats given bupropion 10 mg/kg IP (as in the Porsolt test) and in rats given bupropion 2.7 mg/kg IV (the ID_{50} for inhibition of locus coeruleus firing rate). Plasma concentrations of bupropion were not significantly different between the two procedures (3.0 μmol/L for the Porsolt test versus 2.3 μmol/L for the single-unit experiments). After these doses of bupropion, the plasma concentration of the metabolite 306U73 was 0.4 μmol/L in the Porsolt test and not detectable in blood drawn 5 minutes after IV injection of 2.7 mg/kg, a dose of bupropion that inhibited locus coeruleus firing by 50%.

There were dose-related increases in plasma concentrations of 306U73 5 minutes after increasing IV doses of that compound (Figure 6). The ID_{50} of 306U73 for inhibition of LC firing (1.4 mg/kg IV) resulted in a plasma concentration of 2.0 μmol/L, a value more closely associated with its IC_{50} for inhibition of norepinephrine uptake into rat brain synaptosomes (6.6 ± 3.3 μmol/L) than for inhibition of dopamine (23 ± 8 μmol/L) or serotonin (105 ± 11 μmol/L) uptake (Ferris and Cooper 1993).

Effects of Reserpine Treatment on Monoamine Concentrations in Whole Brain

Table 2 shows the levels of NE, DA, and 5-HT in the brains of a group of rats treated with the dose of reser-

Table 2. Effects of Reserpine (2.5 mg/kg SC) on the Levels of Rat Brain Biogenic Amines

	Controls	Reserpine	% of Control
NE	4.7	0.9	19
DOPAC	0.6	1.2	200
HVA	0.4	1.2	330
DA	6.3	1	16
5HIAA	1.4	3.8	271
5HT	3.2	0.6	19

Values are in picomole/mg of wet weight.
NE = norepinephrine; DOPAC = 3,4 dihydroxyphenylacetic acid; HVA = homovanillic acid; DA = dopamine; 5HIAA = 5-hydroxy indole acetic acid; 5HT = serotonin.

pine used before electrophysiological experiments. Norepinephrine, dopamine, and serotonin were depleted to 19%, 16%, and 19%, respectively, of control values.

DISCUSSION

The acute effects of bupropion in animal antidepressant tests were more closely associated with its actions on the firing rates of noradrenergic locus coeruleus neurons than its effect on midbrain dopamine neurons. Bupropion produced partial inhibition of the activity of dopamine neurons at doses in excess of those that were active in the Porsolt test. The major metabolites of bupropion, 306U73, and 494U73, had no effects on dopamine cell activity at any dose. Additionally, bupropion, 306U73, and 494U73 had no effects on the firing of serotonergic dorsal raphe neurons.

The unexpected electrophysiological effects of the bupropion in the locus coeruleus are at odds with our interpretation of earlier neurochemical studies. Ferris et al. (1981) found that bupropion was nearly twice as potent as an inhibitor of dopamine uptake into striatal synaptosomes than as an inhibitor of norepinephrine uptake into hypothalamic synaptosomes. In rats, bupropion selectively inhibited depletion of dopamine by 6-OHDA, with an ED_{50} of 43 mg/kg IP. Doses over 100 mg/kg were required to antagonize the depletion of norepinephrine produced by the neurotoxin 6-OHDA (Cooper et al. 1980), confirming the relative selectivity of bupropion for the dopamine transporter. Nevertheless, the dose of bupropion that blocked dopamine uptake in vivo was far higher than the doses that were effective in animal tests predictive of antidepressant activity (Porsolt test ED_{50} = 7 mg/kg IP). Whereas the present results do not exclude a possible role for dopamine in the activity of bupropion in animal antidepressant screening tests, they do not support the earlier view that bupropion had a predominantly dopaminergic mechanism in rat.

The causes of the acute reduction of firing rate of locus coeruleus cells produced by bupropion remain to be determined. With regard to indirect mechanisms, inhibition of norepinephrine uptake cannot account for the effect of bupropion on locus coeruleus firing rates because inhibition of NE uptake occurs in vivo at 10-fold higher doses (see Cooper et al. 1980; Ferris et al. 1981; Ferris and Cooper 1993). Furthermore, bupropion is unlike amphetamine in that it does not release norepinephrine or dopamine from synaptosomal preparations (Ferris et al. 1981; Ferris and Cooper 1993). Nevertheless, bupropion does cause a rapid and significant reduction of the firing of locus coeruleus cells that can be reversed by the known α_2 antagonists yohimbine and piperoxane, implying that there is ultimately an activation of α_2 receptors in rat brain. Since bupropion does not have significant affinity for α_1 or α_2 noradernergic receptors, nor does it bind to any other sites that could explain these effects within the context of known relationships between neural systems (Ferris and Cooper, 1993), we considered two other possibilities.

The formation of an active metabolite was considered because the clincial literature has proposed that active metabolites are responsible for the antidepressant activity of bupropion in humans (Perumal et al. 1986; Martin et al. 1990). Both 306U73 and 494U73 display antidepressant activity in mice (Martin et al. 1990). In humans, CSF and plasma concentrations of 306U73 and 494U73 are greater than levels of the parent compound (Perumal et al. 1985; Golden et al. 1988; Ferris and Cooper 1993). In rat electrophysiological tests, however, only 306U73 exhibited a significant inhibition of locus coeruleus firing, which was equipotent with bupropion. 494U73 produced minor effects at the highest nonlethal dose by the IV route.

We next considered that inhibition of norepinephrine uptake by the metabolite 306U73 might be related to the acute electrophysiological effects of bupropion in rat. 306U73 selectively inhibits norepinephrine transport in rat brain (Ferris and Cooper 1993) and plasma concentrations of 306U73 (2.0 μmol/L) obtained 5 minutes after IV injection of the ID_{50} dose of 306U73 for inihibition of locus coeruleus firing were sufficiently high to inhibit the NE transporter. However, the dose of bupropion used in the Porsolt test resulted in plasma levels of 306U73 of only 0.4 μmol/L, which are too low to inhibit the transporter. In addition, 306U73 was not detected after the IV doses of bupropion used in the single-unit experiments. In fact, the only compound detected in rat plasma at the time of electrophysiolgical tests with bupropion following IV injection was bupropion itself. The effects of bupropion on locus coeruleus firing rates in rats after IV injection are difficult to attribute to a metabolite. These effects may better correlate with brain levels of 306U73, which may be significantly high to inhibit the NE transporter.

It is also unlikely that bupropion acted indirectly through another neural system that in turn reduced firing rates of locus coeruleus neurons. Bupropion, 306U73, and 494U73 are not ligands at other major neurotransmitter receptors in mouse, rat (Ferris et al. 1983; Ferris and Cooper 1993), or human brain (Richelson and Nelson 1984; Wander et al. 1986). Furthermore, bupropion inhibits the firing rates of locus coeruleus neurons in brain slices, a preparation with severed afferent connections.

Another possibility was that bupropion exerted effects on locus coeruleus firing rates through an interaction with intracellular norepinephrine. We addressed this issue using pretreatment with reserpine or tetrabenazine to reduce intracellular pools of norepinephrine before recording the electrophysiological effects of bupropion on locus coeruleus neurons. Catecholamine depletion resulted in rightward shifts of the bupropion dose-effect curves without affecting the potency of clonidine. This result indicates that the effects of bupropion on locus coeruleus neurons are mediated indirectly by a reserpine- or tetrabenazine- sensitive pool of intracellular norepinephrine. As previously mentioned, we do not believe that the mechanism of this interaction is easily explainable by classical mechanism of inhibition of NE reuptake or release of catecholamines. This rationale was based on correlations made with plasma concentrations observed in animal models that were not high enough to account for inhibition of NE reuptake. However, brain levels of bupropion and 306U73 could be high enough to block NE reuptake. Preliminary data in rats and mice reveal that bupropion and 306U73 can accumulate in brain at concentrations 10 to 15 times higher than in plasma (unpublished observations). Perhaps brain levels of bupropion or metabolites will better correlate with antidepressant activity than do plasma levels. What is clear is that the electrophysiological and perhaps antidepressant effects of bupropion are dependent on intracaellular pools of at least NE.

The species-dependent metabolism of bupropion complicates the clinical implications of this work. In humans, 306U73 has been identified as a potentially active major metabolite (Perumal et al. 1985). Because 306U73 is an NE transport inhibitor, bupropion may be viewed from a clinical perspective as a noradrenergic agent that acts by forming an NE uptake inhibitor, 306U73. After typical clinical doses of bupropion, the plasma concentration of bupropion is only 0.6 μmol/L, whereas the concentration of 306U73 is 7.2 μmol/L. The IC_{50} of 306U73 for norepinephrine uptake in vitro is 2.3 μmol/L; therefore, there is ample 306U73 available to inhibit norepinephrine transport in depressed patients. The small amount of bupropion present in human CSF and plasma after clinical doses would only augment the primary noradrenergic responses elicited by 306U73. It is also unlikely that the concentrations of bupropion in humans resulting from these doses would produce any acute dopaminergic effects.

Thus, the available information indicates that bupropion predominantly affects the noradrenergic system in rat brain after acute doses. This interaction is difficult to explain by the traditional mechanisms of either release or inhibition of the uptake of NE. A second significant finding is that the pharmacological profile of bupropion differs significantly between humans and rats owing to differences in metabolism. In the rat, little or no 306U73 is present after treatment, so that the noradrenergic behavioral and electrophysiological effects of bupropion result from bupropion. In humans, the effects of bupropion may well result from the large concentration of the metabolite 306U73 in human plasma, which acts to inhibit the NE transporter.

Finally, we have investigated only the acute electrophysiological effects of bupropion and its metabolites in the present study. In patients, the therapeutic effects of antidepressants are generally observed only after several weeks of continuous dosing (Baldessarini 1990). Further studies are in progress to clarify the noradrenergic pharmacology of bupropion after chronic treatment and what role, if any, the brain levels of bupropion and metabolites play in explaining the antidepressant activity of the drug.

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