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RESEARCH PAPER

Comparisons between bupropion and dexamphetamine in a range of *in vivo* tests exploring dopaminergic transmission

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Background and purpose: In the present study we investigated, in a range of *in vivo* tests whether the antidepressant bupropion, and its metabolites shared the dopamine releasing effect of the chemically related dexamphetamine.
Experimental approach: We compared bupropion and dexamphetamine in different neurochemical (microdialysis, DOPAC and HVA contents) and behavioural tests, assessing their effects in animals pretreated with a variety of agents (reserpine, sodium hydroxy-4-butyrate or haloperidol) known to modify dopaminergic transmission.
Key results: In mice, dexamphetamine, like bupropion, increased at low doses and reduced at high doses, locomotor activity. Dexamphetamine restored the locomotor activity in mice made akinetic by either sodium hydroxy-4-butyrate or reserpine, whereas bupropion did not. Moreover, bupropion prevented the dexamphetamine-induced reversal of akinetic effects of reserpine. Haloperidol abolished the locomotor-stimulant effects of dexamphetamine but did not suppress stimulation by bupropion. In microdialysis experiments, in chloral hydrate anesthetized rats, low doses of dexamphetamine ($1\,mg\,kg^{-1}$) markedly increased the extracellular dopamine concentration in striatum (340%), while bupropion ($100\,mg\,kg^{-1}$) produced only a moderate increase (150%). Finally, in rat striatum, as well as in the nucleus accumbens, bupropion increased the effect of haloperidol on DOPAC and HVA concentrations, whereas dexamphetamine reduced these haloperidol effects.
Conclusions and implications: Considering only dopaminergic transmission, our results demonstrated that bupropion and metabolites displayed *in vivo*, as did bupropion *in vitro*, an inhibition of dopamine uptake and, contrast to dexamphetamine, were devoid of dopamine releasing effects.
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Keywords: bupropion; dexamphetamine; dopamine uptake inhibition; locomotion; microdialysis; DOPAC; HVA

Abbreviations: DA, dopamine; DAT, dopamine transporter; DMSO, dimethylsulphoxide; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid

Introduction

Bupropion is an antidepressant, whose efficacy has been suggested in animals (Cooper *et al*., 1980; Steru *et al*., 1987; Martin *et al*., 1990) and demonstrated in humans (Fabre and McLendon, 1978; Zung, 1983). In clinical studies, its antidepressant efficacy appears similar to that of selective serotonin uptake inhibitors or tricyclic antidepressants (Chouinard, 1983; Kavoussi *et al*., 1997). Moreover, bupropion has been more recently proposed as an aid to smoking cessation (Hurt *et al*., 1997).

The mechanism of its antidepressant action seems to depend on inhibition of noradrenaline uptake displayed by its major metabolite, 4-hydroxybupropion (Martin *et al*., 1990; Ascher *et al*., 1995), although the involvement of an increase in the limbic dopaminergic transmission cannot be discounted, as the drug also exhibits inhibition of dopamine (DA) uptake (Cooper *et al*., 1980; Nomikos *et al*., 1992; Learned-Coughlin *et al*., 2003). Furthermore, it has been shown that bupropion is without any effect on 5-hydroxytryptaminergic transmission (Ascher *et al*., 1995) and has no significant affinity for various types of receptors such as α or β adrenoceptors, 5-HT, DA or acetylcholine receptors (Ferris and Beamman, 1983).

In vitro, bupropion is a weak inhibitor of DA uptake as shown in synaptosomes (Sanchez and Hyttel, 1999), but it does not release [^{3}H]DA via human dopamine transporters (DATs) transfected into COS7-cells (Eshleman *et al*., 1994).

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There are a few indications that *in vivo*, the effects of bupropion differ from those of dexamphetamine, such as the prevention by tetrodotoxin of the increase in extracellular DA (using microdialysis), following bupropion (Nomikos *et al*., 1989) but not that following dexamphetamine (Westerink *et al*., 1987b). However, bupropion is chemically related to dexamphetamine, which is well known to release DA. Like dexamphetamine, bupropion stimulates locomotor activity in rodents (Soroko *et al*., 1977) and reduces eating (Zarrindast and Hosseini-Nia, 1988). It produces only mild stereotypies in rats (Nomikos *et al*., 1989; Zarrindast *et al*., 1996). Thus, bupropion could exhibit, *in vivo*, DA-releasing effects and/or only inhibitory effects on DA uptake. Such a distinction is not insignificant in terms of safety for patients as amphetamine-like drugs are known to develop addictive and toxic effects, more marked than those developed by DA uptake inhibitors.

The aim of this study was to determine *in vivo* whether, in terms of DA transmission, bupropion exhibited, like dexamphetamine, DA-releasing properties or whether it is only a DA uptake inhibitor. For this purpose we compared *in vivo*, bupropion and consequently its metabolites with dexamphetamine, in different behavioural tests in which bupropion had not yet been tested or on tests performed in different conditions as these already described. Thus, we studied their effects on locomotion in animals pretreated with various agents modifying dopaminergic transmission such as reserpine, sodium hydroxy-4-butyrate and haloperidol. Bupropion and dexamphetamine were also compared in neurochemical experiments such as microdialysis and the determination of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) contents in the striatum and nucleus accumbens of haloperidol-pretreated rats. The microdialysis experiments were carried out in the striatum of chloral hydrate anaesthetized and acutely implanted rats. Although far from animal physiology, this protocol was chosen to decrease the firing rate of DA neurons (Hamilton *et al*., 1992), allowing a better discrimination between a DA-releasing-effect independent from the electric activity of DA neurons, characteristic of amphetamine-like drugs, and a DA uptake inhibitory action, whose effect on extracellular DA concentration strongly depends on this electric activity.

We conclude that bupropion, although chemically related to dexamphetamine, a well-established releaser of DA, behaved *in vivo* only as a DA uptake inhibitor and was devoid of DA-releasing effects.

Methods

Animals

Male Swiss albino mice CD1 weighing 20–22 g and male Sprague–Dawley rats weighing 200–220 g (Charles River, L' Arbresle, France) were obtained at least 1 week before the beginning of the experiments. The animals were housed in a room maintained at a constant temperature ($21 \pm 1^{\circ}C$), with a regular light cycle (light on between 0700 and 1900 h). Food and water were freely available, except at the time of testing. This study was performed in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No 85–23, revised 1985) and with the principles presented in the European Communities Council Directive of 24 November 1986 (86/609/EEC). Furthermore, the microdialysis protocol was approved by the Regional Ethical Committee for Animal Research (Normandy) with the following numbers: N/03–06–06/06. A total of 167 rats and 395 mice were used in the experiment.

Locomotor activity measurements

A Digiscan actimeter (Omnitech Electronics Inc., Columbus, OH, USA) was used to measure locomotor activities. The individual boxes ($L = 20$; $W = 20$; $H = 30$ cm for mice) were put in a dimly lit room. The horizontal activity was expressed by the total number of beams crossed by animals.

Surgery and microdialysis

Rats were anesthetized with chloral hydrate ($400\,mg\,kg^{-1}$, intraperitoneal) then mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The body temperature was maintained at 37°C throughout the experiment by means of a thermoregulatory heating pad and a rectal probe (Harvard, Holliston, MA, USA). The skull was exposed and a microdialysis probe (membrane: length, 3 mm; outer diameter 0.24 mm; CMA/microdialysis AB, Stockholm, Sweden) was implanted in the right striatum (A/P, +0.5 mm; M/L, −3.0 mm; D/V, −6.5 mm relative to dura and bregma) according to Paxinos and Watson (1986). The probe was connected to a micropump (Stoelting, Wood Dale, IL, USA) and perfused with Ringer's solution (NaCl, 145 mM; KCl, 2.7 mM; $CaCl_2$, 1.2 mM and $MgCl_2$ 1 mM, pH 7.4) at a flow rate of $1.0\,\mu l\,min^{-1}$. After a 2 h equilibration period, successive 20 μl samples were collected in vials containing 10 μl of perchloric acid (0.1 M) and cysteine ($1\,g\,l^{-1}$) and were stored at −80°C until assayed by high-performance liquid chromatography (HPLC). The first four samples were used to determine the basal release of DA. Then dexamphetamine or bupropion or the DA uptake inhibitor GBR12783 was administered subcutaneously (s.c.) and five successive samples were collected. At the end of the experiment, each animal was killed by decapitation and the probe position into the striatum was checked by macroscopic observation after the dissection.

Preparation of samples for determination of neurotransmitter content

Rats were killed by decapitation and their brains were quickly removed. Striata and nucleus accumbens were dissected on ice and homogenized by sonication at high frequency (70 Hz) using a Vibra Cell Sonicator (Sonics and Materials, Danburry, CT, USA) in ice-cold perchloric acid (0.1 M) containing $1\,g\,l^{-1}$ cysteine. Homogenates were centrifuged (12 000 *g*, 10 min at 4°C) and the supernatants were filtered by pressure through 0.45 μm filters (Millipore, Cork, Ireland) before DA, DOPAC and HVA determinations. The pellets were resuspended in NaOH (0.1 M) and used for protein determination, according to Lowry *et al*. (1951).

Determination of DA, DOPAC and HVA

Levels of DA and its metabolites, DOPAC and HVA, were determined using a reverse-phase ion-pair HPLC system with electrochemical detection. The HPLC system consisted of a pump (LC 200; Perkin Elmer, CT, USA) connected to a C18 reversed-phase column (2.0 × 250 mm, 5 μm; Beckman-Coulter, CA, USA) coupled to an electrochemical detector (Decade; Antec Leyden, the Netherlands) with a glassy carbon electrode set at 0.8 V vs an Ag/AgCl reference electrode. The mobile phase consisted of KH_2PO_4, 60 mM; methanol, 50 ml l^{-1}; Pic B7 (sodium heptanesulphonate; Waters, Milford, MA, USA), 5 ml l^{-1} and Na_2EDTA 0.55 mM, at pH 3.6. The sample run time was 45 min, at a flow rate of 0.3 ml min^{-1}. About 20 μl of samples were injected into the system by means of an automatic device (AS 300; Spectra Physics, CA, USA). Identification of the peaks was checked against 100 pg mixtures of external standards and peak heights were quantified with PC integration Borwin Software (JMBS Developments, Le Fontanil, France).

Statistical analysis

The data were expressed as means ± s.e.m. Results were analyzed using one-way analysis of variance (ANOVA) or two-way ANOVA, when appropriate, followed by Student-Newman–Keul's comparisons. When the normality was not reached, the statistical analyses were conducted using a Kruskal–Wallis test. Comparisons between two groups were analyzed by Student's *t*-test. Microdialysis data were expressed as a percentage of basal values evaluated on the first four samples and statistical analysis was performed on these data using a two-way repeated measures followed by a Student-Newman–Keul's test. A *P*-value of <0.05 was considered significant and statistical analyses were performed with SigmaStat (SPSS Inc., Chicago, IL, USA).

Drugs

Dexamphetamine sulphate (CPF, Melun, France) was dissolved in 0.9% NaCl, isotonic saline solution. Bupropion (supplied by GSK, batch no. 1497/4) was dissolved in water for injectable preparations. Reserpine (Sigma, St Louis, MO, USA) was dissolved in dimethylsulphoxide (DMSO) and then diluted in distilled water, and Cremophor EL (BASF, Ludwigshafen, Germany) (final concentration: 5% DMSO and 5% Cremophor EL). Haloperidol (Haldol, Janssen-Cilag, Beerse, Belgium) and the sodium hydroxy-4-butyrate (Gamma-OH, Laboratoires SERB, Paris, France) were diluted in 0.9% NaCl isotonic saline solution. GBR12783 (synthesized by Professor Robba, Caen, France) was dissolved in DMSO and distilled water (final DMSO concentration of 5%).

Results

Effect of increasing doses of dexamphetamine or bupropion on locomotor activity in mice

The administration of dexamphetamine (3 mg kg^{-1}, s.c.) significantly increased the number of crossed beams in the actimeter. At a dose of 6 mg kg^{-1}, the increase in locomotor activity was significantly reduced compared with that developed after the lower dose of 3 mg kg^{-1}. In the same way, bupropion, given s.c, significantly increased locomotor activity (10–40 mg kg^{-1}). At a dose of 80 mg kg^{-1}, bupropion no longer stimulated locomotor activity. Dexamphetamine, at the dose of 3 mg kg^{-1}, induced a greater increase in locomotor activity than bupropion administered at the dose of 40 mg kg^{-1} (Figure 1).

Effect of dexamphetamine and bupropion on akinesia elicited by sodium hydroxy-4-butyrate

Sodium hydroxy-4-butyrate, given i.p. at 375 mg kg^{-1}, induced a complete akinesia in mice (Figure 2a). This akinesia was partially reversed by dexamphetamine (3 mg kg^{-1}, s.c.) but not by bupropion (40 or 80 mg kg^{-1}, s.c.) (Figure 2b).

Effect of haloperidol on dexamphetamine or bupropion stimulant effect

Haloperidol (50–200 μg kg^{-1}, i.p.) significantly reduced the locomotor activity of mice at the two higher doses (100 and 200 μg kg^{-1}). Over the same range of doses, haloperidol dose-dependently reduced the stimulant effect of dexamphetamine (3 mg kg^{-1}, s.c.) but it did not modify the stimulant effect of bupropion (5 and 20 mg kg^{-1}, s.c.) in mice (Figure 3).

Effect of dexamphetamine and bupropion on akinesia elicited by reserpine

Reserpine (4 mg kg^{-1}, s.c., 5 h 40 min before the test) induced a complete akinesia in mice. Administration of bupropion (5, 10, 20 mg kg^{-1}, s.c.), 5 h after reserpine and 40 min before

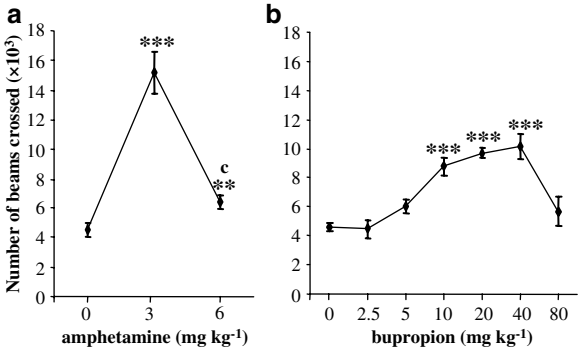

Figure 1 Effect of increasing doses of dexamphetamine or bupropion on locomotor activity. Mice were injected s.c. with saline (0) or increasing doses of dexamphetamine (**a**) or bupropion (**b**), 10 min before their introduction into the actimeter. The horizontal locomotor activity was measured during 40 min and expressed as the number of crossed beams. Means ± s.e.m. of 10–21 animals per group are shown. One-way ANOVA followed by a Student-Newman–Keul's test indicate a statistically significant stimulant locomotor effect of dexamphetamine ($F(2,28) = 41.563$, $P<0.001$) as well as of bupropion ($F(6,74) = 16.782$, $P<0.001$). $^{**}P<0.01$ and $^{***}P<0.001$ as compared with solvent controls. $^{c}P<0.001$ for the comparison between dexamphetamine 3 mg kg^{-1} vs dexamphetamine 6 mg kg^{-1}.

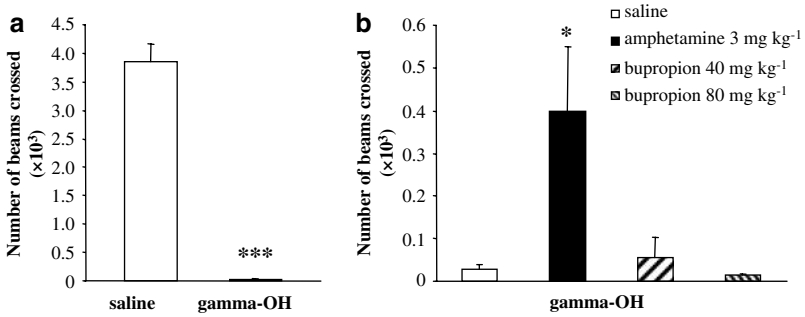

Figure 2 Effect of dexamphetamine and bupropion on the akinesia induced by sodium hydroxy-4-butyrate (Gamma-OH). Mice were pretreated i.p. with saline or sodium hydroxy-4-butyrate (375 mg kg^{-1}) 10 min before solvent (s.c.), dexamphetamine (3 mg kg^{-1}, s.c.) or bupropion (40 and 80 mg kg^{-1}, s.c.) injection. About 10 min later, mice were introduced into the actimeter. Their locomotor activity was measured during 30 min and expressed as the number of crossed beams. Means ± s.e.m. of 14–15 animals per group are shown. Student's *t*-test indicates a significant difference between control mice and sodium hydroxy-4-butyrate-treated mice ($P<0.001$) (**a**). Kruskal–Wallis test indicates a significant effect of dexamphetamine (3 mg kg^{-1}) ($P<0.05$) but no effect of bupropion (40 and 80 mg kg^{-1}) on locomotor activity of sodium hydroxy-4-butyrate-pretreated mice ($P>0.05$) (**b**). *$P<0.05$ and ***$P<0.001$.

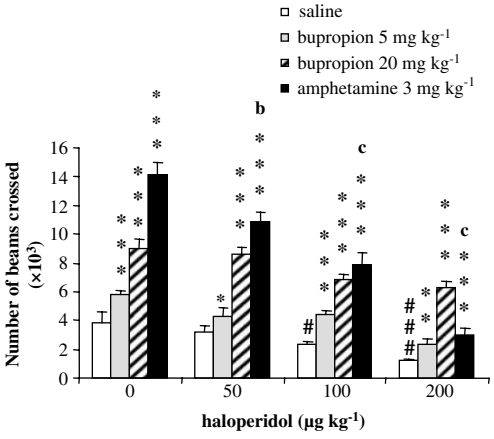

Figure 3 Effect of haloperidol on dexamphetamine- or bupropion-induced motor stimulant effect. Mice were pretreated i.p. with saline or haloperidol (50, 100 or 200 μg kg^{-1}); 20 min later, mice were treated s.c. with dexamphetamine (3 mg kg^{-1}) or bupropion (5 and 20 mg kg^{-1}). About 10 min later, mice were introduced into the actimeter. Their locomotor activity was measured during 40 min and expressed as the number of crossed beams. Means ± s.e.m. of nine animals per group are shown. Two-way ANOVA followed by a Student-Newman–Keul's test indicate a significant interaction between haloperidol and dexamphetamine 3 mg kg^{-1} ($F(3,64)=10.459$; $P<0.001$) but no interaction between haloperidol and bupropion 5 and 20 mg kg^{-1} ($F(6,96)=1.578$; $P>0.05$). $^{\#}P<0.05$; $^{\#\#\#}P<0.001$ as compared with the saline–saline group. *$P<0.05$; **$P<0.01$ and ***$P<0.001$ as compared with their respective haloperidol–saline group. $^{b}P<0.01$ and $^{c}P<0.001$ for the comparison between mice treated by dexamphetamine or bupropion alone with mice treated by dexamphetamine or bupropion associated with the different doses of haloperidol.

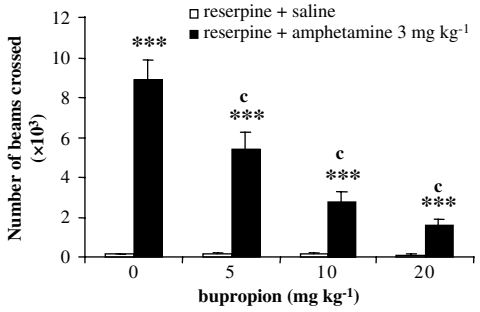

Figure 4 Effect of bupropion or dexamphetamine or their association on reserpine-induced akinesia. Mice were pretreated s.c. with reserpine 4 mg kg^{-1}, 5 h before s.c. administration of saline or bupropion (5, 10 or 20 mg kg^{-1}); 30 min later and 10 min before introduction into the actimeter, they received s.c. saline or dexamphetamine 3 mg kg^{-1}. Their locomotor activity was then measured during 40 min and expressed as the number of beams crossed. Means ± s.e.m. of 9–10 animals per group are shown. Two-way ANOVA followed by Student-Newman–Keul's test indicate in reserpine-pretreated mice, a significant locomotor stimulant effect of dexamphetamine ($P<0.001$), no effect of bupropion ($P>0.05$ for all tested doses) and a significant interaction between bupropion and dexamphetamine in reserpine-pretreated mice ($F(3,70)=16.806$; $P<0.001$). ***$P<0.001$ as compared with their respective reserpine–saline group. $^{c}P<0.001$ for the comparison between mice treated by reserpine and dexamphetamine with mice treated by reserpine and dexamphetamine associated with the different doses of bupropion.

the test, did not reverse reserpine-induced akinesia (Figure 4). Conversely, dexamphetamine (3 mg kg^{-1}, s.c.) administered 30 min after saline and 10 min before the test in reserpine-pretreated mice increased locomotor activity ($P<0.001$). However, when dexamphetamine was administered 30 min after bupropion in reserpine-pretreated mice, bupropion, from a dose of 10 mg kg^{-1}, opposed dose-dependently the reversal by dexamphetamine of the akinesia elicited by reserpine (Figure 4).

Effect of dexamphetamine, bupropion or GBR12783 on extracellular concentration of DA in rat striatum

In rats anesthetized by chloral hydrate (400 mg kg^{-1}, i.p.), dexamphetamine (1, 2.5 and 7.5 mg kg^{-1}, s.c.) produced a dose-dependent increase in striatal DA levels. The maximum effects (339, 270 and 532% of the basal levels, respectively) were observed between 40 and 60 min after dexamphetamine s.c. injection and the increase in DA concentration remained at a higher level until the end of the experiment (160 min) (Figure 5a). In a previous experiment, performed under similar conditions, we observed that the specific DA uptake inhibitor, GBR 12783 (5 and 15 mg kg^{-1}, s.c.), did not increase the extracellular level of DA (Figure 5b). Finally,

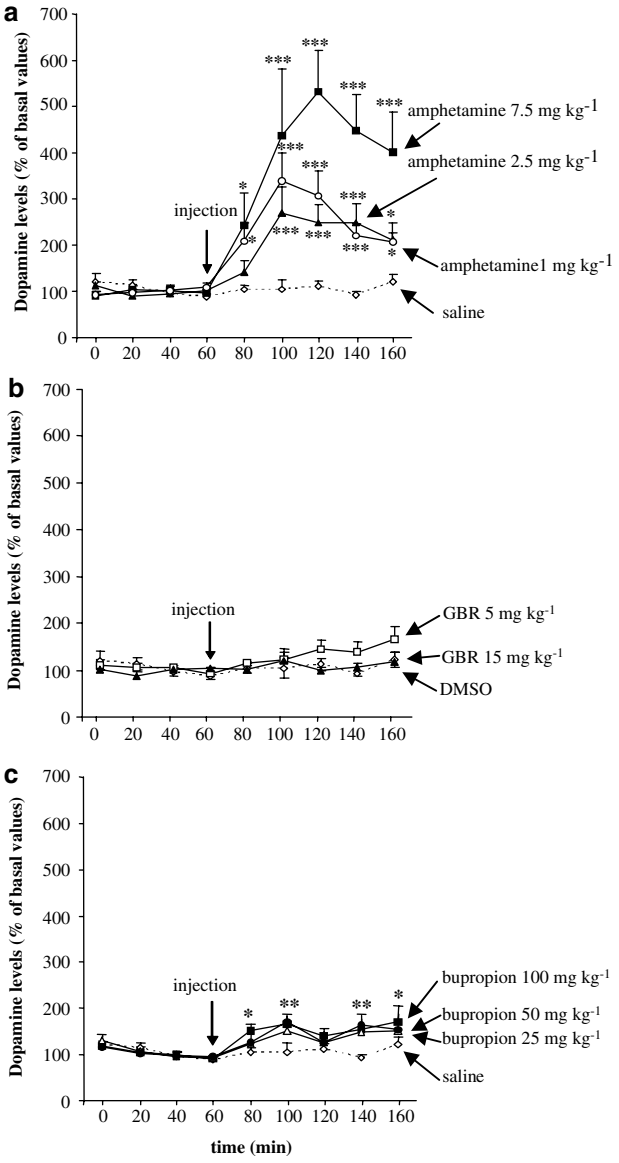

Figure 5 Effect of dexamphetamine, GBR12783 or bupropion on extracellular DA concentration in the striatum of chloral hydrate-anesthetized rats. A microdialysis probe inserted into the right striatum and perfused with Ringer's solution at a flow rate of 1 μl min^{-1}. After a 2-h equilibration period, 20 μl of samples were collected and analysed by HPLC to measure DA concentrations. After collection of the first four samples, used to determine the basal release of DA, an injection of dexamphetamine (1, 2.5 and 7.5 mg kg^{-1}) or GBR 12783 (5 and 15 mg kg^{-1}) or bupropion (25, 50 and 100 mg kg^{-1} s.c.) was given. The basal DA level in dialysates, in the first four samples, was 25.9 $\pm$ 4.3 fmol min^{-1}. Data are expressed as percentages of basal DA concentration in dialysates. Means $\pm$ s.e.m. of 5–6 rats per group are shown. Two-way repeated measures ANOVA followed by a Student-Newman–Keul's test indicate that dexamphetamine produced a significant increase in DA level (interaction (treatment $\times$ time): F (24,160) = 5.253; $P<0.001$) (**a**). GBR12783 did not increase DA level (interaction (treatment $\times$ time): F (16,120) = 0.928; $P>0.05$) (**b**). Only the 100 mg kg^{-1} dose of bupropion was found to increase the DA at a level statistically significant (interaction (treatment $\times$ time): F (8,80) = 2.114; $P=0.044$) (**c**). *$P<0.05$, **$P<0.01$ and ***$P<0.001$, as compared with solvent controls.

bupropion (25, 50 and 100 mg kg^{-1}, s.c.) induced a moderate increase in DA levels throughout the experiments, independent of the dose (Figure 5c). Only the dose of 100 mg kg^{-1} was found to increase the extracellular DA concentration, compared with that of the control animals. This increase was statistically significant 20 min after the bupropion injection (152% of the basal levels) and remained at this level until the end of the experiment.

Effect of bupropion and dexamphetamine on HVA and DOPAC concentration in striatum and nucleus accumbens of haloperidol-pretreated rats

In this experiment, haloperidol (450 μg kg^{-1}, i.p.) increased the DOPAC/DA and the HVA/DA ratios, both in the striatum and in the nucleus accumbens.

In the striatum, bupropion (10, 20, 40 and 80 mg kg^{-1}, s.c.) did not modify the DOPAC/DA or the HVA/DA ratios, when given alone (Figure 6a). Given to haloperidol pretreated animals, bupropion potentiated dose-dependently the effects of haloperidol on these two ratios (Figure 6a). Dexamphetamine (1, 2.5 and 7.5 mg kg^{-1}, s.c.) given alone also did not modify the DOPAC/DA and HVA/DA ratios. However, in contrast to bupropion, it reduced the increase of these ratios elicited by haloperidol (Figure 6b).

In the nucleus accumbens, neither bupropion nor dexamphetamine modified, by themselves, the DOPAC/DA and the HVA/DA ratios. In this structure, bupropion from the dose of 40 mg kg^{-1}, as in the striatum, potentiated the haloperidol increasing effect on the DOPAC/DA ratio and at the higher dose of 80 mg kg^{-1}, on the HVA/DA ratio (Figure 6c). Dexamphetamine still opposed the increasing effect of haloperidol but, in this tissue, only for the DOPAC/DA ratio (Figure 6d).

Discussion

The locomotor stimulant effect of bupropion has already been described in rodents (Soroko *et al*., 1977). In the present experiments, we observed that this effect of bupropion in mice, like that of dexamphetamine, was biphasic. Thus, the maximal stimulation of horizontal locomotion was obtained at 40 mg kg^{-1}, whereas it disappeared at 80 mg kg^{-1}. As high doses of bupropion and dexamphetamine had been shown to produce stereotypies in rats (Nomikos *et al*., 1989), these stereotypies might oppose the stimulant locomotor effect of both drugs at the highest tested dose. The locomotor stimulant effect of bupropion (40 mg kg^{-1}) was clearly lower than that of dexamphetamine (3 mg kg^{-1}). This difference may be compared with the increase in the extracellular DA concentration obtained in our microdialysis experiments. In a microdialysis study performed in the striatum of conscious rats, Nomikos *et al*. (1989) observed that bupropion, at doses ranging from 10 to 100 mg kg^{-1}, increased the extracellular concentration of DA in a dose- and time-dependent manner, with a maximal increase of 543%. Because of this strong increase and to make a clear distinction between a possible DA-releasing effect (typical of amphetamine-like drugs, independent of neuronal firing rate) and a potential DA

uptake inhibitory effect of bupropion, we chose to carry out our microdialysis experiments in rats anaesthetized with chloral hydrate and acutely implanted, in order to compare bupropion and dexamphetamine. Indeed, we observed that the increase in extracellular DA concentration obtained in the striatum of anaesthetized rats by a low dose of dexamphetamine (1 mg kg^{-1}) was by far more marked than that induced either by the pure DA uptake inhibitor GBR12783 (5–15 mg kg^{-1}) or by bupropion, even at the dose of 100 mg kg^{-1}. This difference results from the chloral hydrate anaesthesia administered to our rats as chloral hydrate reduces the firing rate of dopaminergic nigro-striatal neurons and, consequently, the DA release by striatal terminals (Hamilton *et al*., 1992). This DA released from neurons is normally removed from the extracellular space by the DA uptake system. Bupropion, by inhibiting DA uptake, leads to an accumulation of DA, which is measured as an increase in extracellular DA concentration. This effect is diminished when DA release is decreased, consequent to the reduction by chloral hydrate of the firing rate of nigro-striatal dopaminergic. This explanation is compatible with the results of Nomikos *et al*. (1989, 1990), who found that the increase in extracellular DA concentration elicited by bupropion in the striatum of conscious rats was blocked by tetrodotoxin, establishing that this effect was dependent on action potentials, that is, on neuronal activity. In this way, our results, as well as those obtained by Nomikos *et al*. (1989, 1990), clearly demonstrate that bupropion and dexamphetamine affect DA transmission differently, as the effect of dexamphetamine, which releases DA independently of the neuronal firing rate, remained elevated in spite of the chloral hydrate anaesthesia (this study) and was unaffected by tetrodotoxin (Westerink *et al*., 1987b; Nomikos *et al*., 1990).

The akinesia induced by sodium hydroxy-4-butyrate is due to the inhibition of the tonic electric activity of nigro-striatal and meso-accumbic dopaminergic neurons (Engberg and Nissbrandt, 1993; Erhardt *et al*., 1998). Amphetamine-like agents that release DA independently of the firing rate of these neurons reverse this akinesia (Duterte-Boucher and Costentin, 1991). In the present study, bupropion, in contrast to dexamphetamine, failed to reverse this akinesia, indicating a lack of DA-releasing effect. This is also corroborated by the inability of bupropion to reverse the reserpine-induced akinesia, whereas dexamphetamine was effective in this respect. As a matter of fact, reserpine inhibits the vesicular monoamine transporter type 2, preventing the

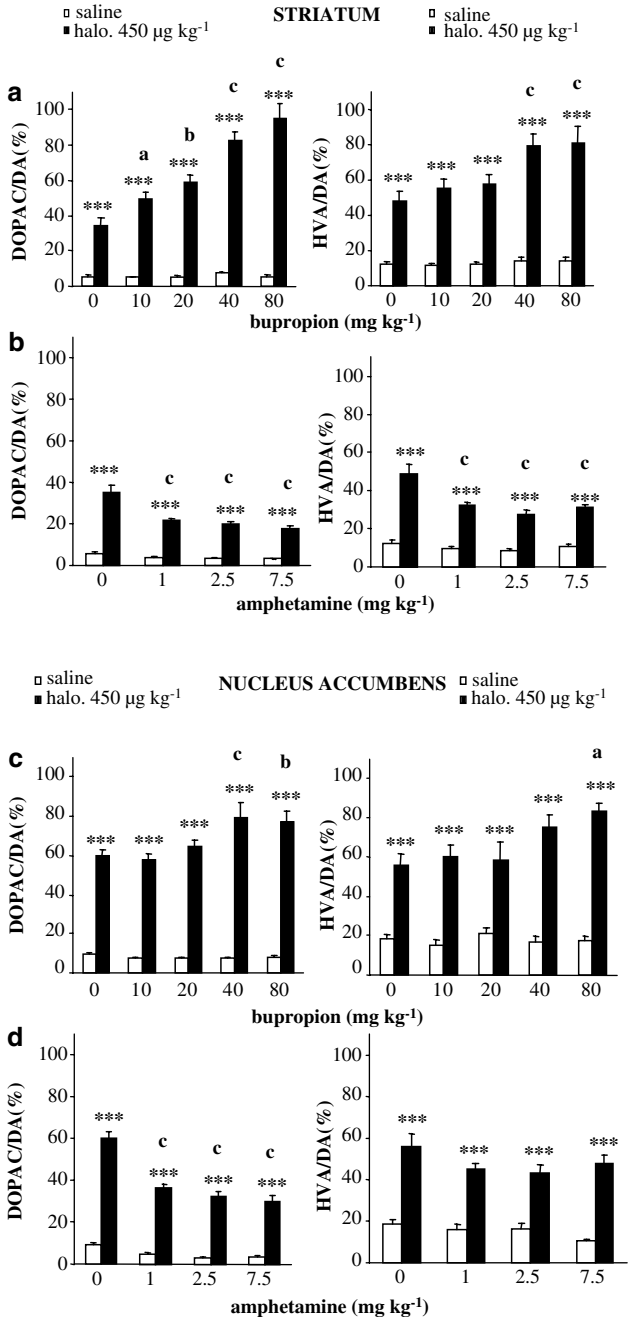

Figure 6 Effect of dexamphetamine and bupropion on the DOPAC/DA and the HVA/DA ratios in striatum and nucleus accumbens of haloperidol-pretreated rats. Rats were pretreated i.p. with saline (white columns) or haloperidol (450 μg kg^{-1}) (black columns). About 30 min later they were treated s.c. with dexamphetamine (1, 2.5 and 7.5 mg kg^{-1}) or bupropion (10, 20, 40 and 80 mg kg^{-1}). And 30 min after this injection, rats were sacrificed and DA, DOPAC and HVA concentrations were determined in the striata and accumbens nuclei of each rat. The data are expressed as the DOPAC/DA and HVA/DA ratios. Means$\pm$s.e.m. of seven rats per group are shown. Two-way ANOVA followed by a Student-Newman–Keul's test indicate, in the striatum, a significant interaction between haloperidol and bupropion (F (4,60) = 6.217; $P<0.001$ for the DOPAC/DA ratio and F (4,60) = 2.790; $P=0.034$ for the HVA/DA ratio) (**a**) and a significant interaction between haloperidol and dexamphetamine (F (3,48) = 3.137; $P=0.034$ for the DOPAC/DA ratio and F (3,48) = 2.857; $P=0.047$ for the HVA/DA ratio) (**b**). In nucleus accumbens, two-way ANOVA followed by a Student-Newman–Keul's test indicate a significant interaction between haloperidol and bupropion (F (4,60) = 4.666; $P=0.002$ for the DOPAC/DA ratio and F (4,60) = 2.787; $P=0.034$ for the HVA/DA ratio) (**c**) and a significant interaction between haloperidol and dexamphetamine for the DOPAC/DA ratio (F (3,48) = 15.107; $P<0.001$) but not for the HVA/DA ratio (**d**). ***$P<0.001$ as compared with solvent controls. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ for the comparison between haloperidol-pretreated rats and haloperidol-pretreated rats injected with the different doses of dexamphetamine or bupropion.

vesicular storage of monoamines (Erickson *et al*., 1992). Reserpine-induced akinesia results from a suppression of dopaminergic transmission, as dopaminergic neurons are no longer able to release DA. Therefore, under these conditions, the DA uptake inhibitors are unable to increase the extracellular, synaptic concentration of the amine. On the other hand, amphetamine-like compounds, by releasing the newly synthesized cytosolic pool of DA, restore the stimulation of post-synaptic dopaminergic receptors and thereby locomotor activity. Moreover, we observed that bupropion, co-administered with dexamphetamine, inhibits the dexamphetamine-induced reversion of the akinesia elicited by reserpine. This may be explained by the fact that bupropion acts by blocking the DAT, preventing both neuronal uptake of amphetamine and the subsequent release of DA mediated by the transporters acting in a reverse manner (Garcia de Mateos-Verchere *et al*., 1998; Zaczek *et al*., 1991; Simon *et al*., 1995; Jones *et al*., 1998a).

Haloperidol did not prevent the stimulant effect of bupropion on motor activity. We have previously observed such a lack of antagonism when haloperidol was used with the DA uptake inhibitors GBR12783 (Duterte-Boucher *et al*., 1990) or oxolinic acid (Garcia de Mateos-Verchere *et al*., 1998). The blockade by haloperidol of DA D2 autoreceptors in somato-dendritic locations increases the firing rate of nigro-striatal and meso-accumbic dopaminergic neurons (Bunney *et al*., 1973), and thereby enhances in terminals of DA neurons, DA synthesis and synaptic release from the vesicular stores as well as increasing DA turnover (Anden *et al*., 1971) and thus production of DOPAC and HVA (Westerink and Korf, 1975; Shore, 1976; Kuczenski, 1980). Consequently, blockade of DAT by bupropion and the simultaneous increase of DA release induced by haloperidol, dramatically increased the extracellular DA concentration. At the level of post-synaptic DA receptors, these very high concentrations of DA could compete successfully with haloperidol, thereby maintaining the excito-locomotor effect. On the contrary, the dexamphetamine-stimulant effect was easily reversed by haloperidol, as the DA release induced by dexamphetamine is independent of presynaptic regulation of the neuronal activity by DA autoreceptors. Thus, as dexamphetamine induces release of a definite quantity of DA, blockade of post-synaptic DA receptors by haloperidol dose-dependently reduces its stimulant locomotor effect.

We observed that haloperidol increased the DOPAC/DA ratio, as well as the HVA/DA ratio, both in the striatum and the nucleus accumbens of rats. Interestingly, when bupropion was administered in haloperidol-pretreated rats, we found that the increase in DOPAC/DA and HVA/DA was much greater than in animals treated with haloperidol alone. Similar results were obtained with the selective DA uptake inhibitors amfonelic acid and GBR12783 (Miller and Shore, 1982; Boulay *et al*., 1996). Westerink *et al*. (1987a) explain this synergy by an impulse-dependent mechanism. The blockade of DAT by a DA uptake inhibitor might induce a compensatory increase in DA synthesis and thereby an increase in DOPAC formation by intraneuronal monoamine oxidase (MAO). This hypothesis is supported by recent studies performed in mice lacking the DAT, which show a twofold increase in DA synthesis as compared with control mice (Jones *et al*., 1998b; Benoit-Marand *et al*., 2000). Under normal conditions, this increase in DA and DOPAC formation is counteracted by the activation of D2 autoreceptors, stimulated by the increased DA concentration in the synaptic cleft, subsequent to the inhibition of DA uptake. The co-administration of the D2 DA receptor antagonist haloperidol, with a DA uptake inhibitor, should strongly stimulate DA synthesis and DOPAC formation. Alternatively, it may be also suggested that the high concentration of DA, established in the synaptic cleft by combining haloperidol and a DA uptake inhibitor, could participate in the increased DOPAC formation in the surrounding astroglial cells. In these cells, provided with MAO and catechol-*O*-methyl transferase (COMT) activities (Naudon *et al*., 1992), DA uptake is carried out by the so-called 'extraneuronal monoamine transporter' (Wu *et al*., 1998) which displays a very different pharmacological profile of inhibition as, for instance, it is insensitive to either cocaine (Eisenhofer, 2001) or the selective and potent neuronal DA uptake inhibitor GBR12935 (Takeda *et al*., 2002). Finally, this high DOPAC level leads to an increase in HVA formation, as HVA is formed predominantly from DOPAC by COMT (Westerink, 1979). On the other hand, when dexamphetamine was administered in haloperidol-pretreated rats, a partial reversal of the haloperidol-induced increase of DA metabolism was observed. Four effects of dexamphetamine could explain these results: (i) DA release from nigral dendritic varicosities, which competes with haloperidol on somatodendritic dopaminergic autoreceptors, decreasing the activation of the firing rate of dopaminergic neurons operated by haloperidol; (ii) blockade of the neuronal DAT, which prevents the reuptake of released DA into the neuron and protects the amine from inactivation by the intra-neuronal MAO; (iii) blockade of the extraneuronal monoamine transporter preventing the DA uptake into astroglial cells and its metabolism by astroglial MAO and COMT (Wu *et al*., 1998); and (iv) a direct inhibition of MAO (Green and El Hait, 1978). Taken together, these effects lead to a decrease in DOPAC/DA ratio and in HVA/DA ratio, relative to those observed in response to haloperidol alone. In this respect, bupropion and dexamphetamine act in exactly opposite ways.

In conclusion, all our experiments were performed *in vivo* and thereby involved both bupropion and its major metabolites. They indicate, in a coherent manner, whatever the variables measured, that, as reported previously *in vitro* by other authors, bupropion behaved as a DA uptake inhibitor and not like the DA releaser, dexamphetamine. Such a difference could have consequences for the toxic and addictive properties of this drug, as these properties are known to be more marked in the amphetamines.

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Conflict of interest

The authors state no conflict of interest.

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