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Research Paper

Effects of co-administration of bupropion and nicotine or D-amphetamine on the elevated plus maze test in mice

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Abstract

Objectives A variety of abused drugs, including psychostimulants, can modulate the expression of anxiety. Although the effect of nicotine and D-amphetamine on anxiety-related behaviour in animal models has been investigated, the mechanisms underlying the anxiogenic or anxiolytic actions of these drugs have not been clarified. Bupropion is an antidepressant drug which may alleviate some symptoms of nicotine withdrawal, although its effects on anxiety are not clear. We have investigated the effect of nicotine and D-amphetamine on anxiety in the elevated plus maze test in mice.

Methods We examined the influence of acute administration of nicotine (0.1 mg/kg, s.c.) and D-amphetamine (2 mg/kg, i.p.) on anxiety level. We then evaluated the anxiety-related response after subchronic injection of both psychostimulants, including crossover effects. For this purpose, nicotine (0.1 mg/kg, s.c.) was administered daily for six days, and on the seventh day mice were challenged with nicotine (0.1 mg/kg, s.c.) or D-amphetamine (2 mg/kg, i.p.). A distinct group of mice was pretreated with D-amphetamine (2 mg/kg, i.p., 8 days), and subjected to D-amphetamine (2 mg/kg, i.p.) or nicotine (0.1 mg/kg, s.c.) challenge on the ninth day. Moreover, we investigated acute and subchronic effects of co-administration of bupropion (5, 10 and 20 mg/kg; i.p.) and nicotine or D-amphetamine.

Key findings We observed that acute anxiogenic effects of nicotine and D-amphetamine as well as the development of tolerance and cross-tolerance to their effects were blunted by a pretreatment with a nonactive dose of bupropion (5 mg/kg, i.p.).

Conclusions These results demonstrated that similar neural mechanisms were involved in the regulation of nicotine and D-amphetamine anxiety-like behaviour in mice. The results have provided new findings to support the use of bupropion in the treatment of nicotine and/or amphetamine addiction.

Keywords anxiety; bupropion; elevated plus maze test; psychostimulant

Introduction

The atypical antidepressant bupropion was the first non-nicotine pharmacotherapy for smoking cessation. In humans, bupropion reduces discomfort and craving associated with smoking cessation, while in rodent models of nicotine dependence this drug attenuates nicotine withdrawal syndrome.[1–3] As yet, the precise pharmacological mechanism of action of this drug remains uncertain. It is known to be a weak but relatively selective inhibitor of the dopamine and noradrenaline transporters, and to act as a noncompetitive antagonist for neuronal nicotinic cholinergic receptors (nAChRs).[1,4]

A variety of abused drugs have been found to be capable of modulating the expression of anxiety. For instance, despite reward and cognitive enhancement, anxiolysis is one of the main effects underlying nicotine and/or amphetamine dependence.[5] It has been suggested that the negative affective (e.g. anxiogenic) effects associated with nicotine or D-amphetamine withdrawal promote continued drug use and contribute to the high relapse rate.[6] In animal models, both nicotine and D-amphetamine can affect anxiety in different ways. For instance, it has been shown that nicotine can be anxiogenic, anxiolytic or have no effect on anxiety level.[7–9] Concerning amphetamine, an anxiogenic-like effect on the plus maze test has been shown in rats and mice.[10–11] However, some authors reported that amphetamine failed to alter signs of anxiety in mice or produced anxiolytic-like action in rats.[12,13] These apparently discordant data could be attributable to the differences in dose, time between injections and testing, route of administration, strain differences or behavioural test used.[14,15]

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The complex influence of psychostimulants on anxiety-related effects mentioned above can be associated with enhanced release of different neurotransmitters, and engages a variety of brain structures. It is well established that activation of central nAChRs by nicotine, especially those situated predominantly presynaptically, modulates release not only of acetylcholine, but also of noradrenaline, dopamine, γ-aminobutyric acid (GABA), serotonin and glutamate, which play a role in the pathogenesis of anxiety.[16,17] In turn, it is well established that amphetamine increases dopamine, noradrenaline and serotonin neurotransmission by acting on monoamine transporters, causing an increase in the cytoplasmic levels of monoamines, and leading to an increase in their release from the terminals.[18] Anatomically, the behavioural expression of anxiety is associated with a set of interrelated limbic and cortical structures, such as the septo-hippocampal system, amygdala, hypothalamus and periaqueductal grey matter of the midbrain.[17]

The primary objective of this study was to investigate further the effect of nicotine and D-amphetamine on anxiety in the elevated plus maze test in mice.[7,10–13,19–21] In this respect, we evaluated the anxiety-related response after acute and then subchronic injection of nicotine or D-amphetamine to determine if there were any crossover effects between these drugs. The range of doses was selected taking into account studies of effects of nicotine and D-amphetamine on anxiety in mice, and behavioural data obtained in our laboratory.[8,9,19,21,22] Additionally, we have investigated the influence of bupropion, an antidepressant and antismoking agent, on the acute and subchronic anxiety-related action of both psychostimulants as well as on the acquisition of cross-tolerance between nicotine and D-amphetamine (or D-amphetamine and nicotine). The results of these studies have been discussed in the context of influence of nicotine and D-amphetamine treatment on anxiety-related responses. Moreover, we considered that understanding the behavioural effects of bupropion and its combination with nicotine or, even more interestingly, with D-amphetamine, may aid in improved knowledge of how this antidepressant promotes smoking cessation, and could be important in designing more effective treatments for psychostimulant-related addiction.

Materials and Methods

Animals

The experiments were carried out on naive male Swiss mice (Farm of Laboratory Animals, Warszawa, Poland) weighing 20–25 g at the beginning of the experiments. The animals were maintained under standard laboratory conditions (12-h light/dark cycle, room temperature 21 ± 1°C) with free access to tap water and laboratory chow (Bacutil, Motycz, Poland). The animals were adapted to the laboratory conditions for at least one week. Each experimental group consisted of eight to ten animals. All experiments were carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Council Directive for Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC), and approved by the local ethics committee.

Drugs

The compounds tested were: (–)-nicotine hydrogen tartrate (Sigma, St Louis, Missouri, US), D-amphetamine sulfate (Sigma, St Louis, Missouri, US) and bupropion hydrochloride (Sigma, St Louis, Missouri, US). All compounds were dissolved in saline (0.9% NaCl) and refer to the salt form. All agents were administered intraperitoneally (i.p.) or subcutaneously (s.c.) in a volume of 10 ml/kg. Control groups received saline injections at the same volume and by the same route.

Elevated plus maze procedure

Anxiety responses were measured in the elevated plus maze test. The procedure was similar to the method of Lister[12]. The experimental apparatus was shaped like a 'plus' sign and consisted of a central platform (5 × 5 cm), two open arms (30 × 5 cm) and two equal-sized closed (30 × 5 × 15 cm) arms opposite to each other. The maze was made of dark Plexiglas, elevated to a height of 50 cm above the floor and illuminated by a dim light. The test consisted of placing a mouse in the central platform facing an enclosed arm and allowing it to freely explore the maze for 5 min. Entry into one arm was defined as the animal placing all four paws past the line dividing the central square from the open arms. The test arena was wiped with a damp cloth after each trial. The number of entries into the open and closed arms and the time spent in open arms were measured by an observer blind to the drug treatment. Anxiolytic activity was indicated by increases in time spent in open arms or in the number of open arm entries; anxiogenic effects were characterized by decreases in these measures. The percentage of time spent in the open arms was calculated, as was the percentage number of open arm entries. Additionally, the number of entries into the closed arms was recorded as an indicator of motor activity of animals in this test.

Treatment

Procedure and doses used have been chosen according to published data.[21]

Effects of acute injection of nicotine and D-amphetamine

During acute treatment, the animals were allocated to the following drug groups: nicotine (0.1 mg/kg, s.c.), D-amphetamine (2 mg/kg, i.p.), bupropion (5, 10 and 20 mg/kg, i.p.) or saline. Mice from each group were tested 30 min after injection. The exploratory behaviour in the maze was recorded for 5 min.

Effects of subchronic injections of nicotine and D-amphetamine

Animals were randomly allocated to receive eight daily intraperitoneal injections of D-amphetamine (2 mg/kg) or saline. On the ninth day, these animals were subjected to D-amphetamine (2 mg/kg, i.p.), nicotine (0.1 mg/kg, s.c.) or saline (for a control group), and were tested 30 min after this

last injection. Additionally, another group of animals was randomly allocated to receive six daily subcutaneous injections of nicotine (0.1 mg/kg) or saline. On the seventh day, mice were treated with nicotine (0.1 mg/kg, s.c.), D-amphetamine (2 mg/kg, i.p.) or saline (for a control group), and were tested 30 min after this last injection. The number of entries into open and closed arms and the time spent in open arms were measured 30 min after nicotine or D-amphetamine challenge injection on the test day. These experimental procedures mentioned above were performed to see if tolerance and cross-tolerance to the anxiogenic effect of D-amphetamine and nicotine developed after the longer pretreatment period.

Influence of bupropion on the acute and subchronic effects of nicotine and D-amphetamine

Distinct groups of mice were injected with bupropion (5 mg/kg, i.p.), 15 min before an acute or every subchronic nicotine, D-amphetamine or saline injection. On the test day, these mice were challenged with 0.1 mg/kg nicotine, 2 mg/kg D-amphetamine or saline, as described above (for details see Tables 1 and 2), and their exploratory behaviour in the maze was recorded 5 min after injection.

Statistical analysis

The data are expressed as the means ± standard error of the mean (SEM). The statistical analyses for each measure were performed using one-way analysis of variance with drug treatment on the test day as the dependent factor. Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate. The confidence limit of $P < 0.05$ was considered statistically significant.

Results

Effects of acute injection of bupropion

Acute intraperitoneal doses of bupropion (10 and 20 mg/kg) significantly increased the percentage of time spent on the open arms ($P < 0.001$) and the percentage of open arm entries ($P < 0.05$ for 20 mg/kg), indicating an anxiolytic effect as compared with saline-treated mice (Figure 1). The lowest dose of bupropion (5 mg/kg) did not cause any effect in this paradigm, and this dose was chosen for all subsequent tests.

Effects of acute injection of nicotine and D-amphetamine

Figure 2 shows that in the control saline-treated animals an acute subcutaneous dose of nicotine (0.1 mg/kg) as well as an acute intraperitoneal dose of D-amphetamine (2 mg/kg) significantly decreased the percentage of time spent in open arms, indicating their anxiogenic effect (nicotine: $P < 0.001$ for open-arm time and open-arm entries; D-amphetamine: $P < 0.001$ for open-arm time, $P < 0.05$ for open-arm entries).

Table 1 Sequence of subchronic treatment used

Treatment	Day 1–6	Day 7
Acute nicotine	Saline (i.p.) + saline (s.c.)	Nicotine (0.1 mg/kg, s.c.)
Subchronic nicotine	Saline (i.p.) + nicotine (0.1 mg/kg, s.c.)	Nicotine (0.1 mg/kg, s.c.)
Influence of bupropion on the effect of subchronic nicotine	Bupropion (5 mg/kg, i.p.) + nicotine (0.1 mg/kg, s.c.)	Nicotine (0.1 mg/kg, s.c.)
Acute amphetamine	Saline (i.p.) + saline (s.c.)	Amphetamine (2 mg/kg, i.p.)
Development of cross-tolerance between nicotine and amphetamine	Saline (i.p.) + nicotine (0.1 mg/kg, s.c.)	Amphetamine (2 mg/kg, i.p.)
Influence of bupropion on the development of cross-tolerance between nicotine and amphetamine	Bupropion (5 mg/kg, i.p.) + nicotine (0.1 mg/kg, s.c.)	Amphetamine (2 mg/kg, i.p.)

See Figure 3.

Table 2 Sequence of subchronic treatment used

Treatment	Day 1–8	Day 9
Acute amphetamine	Saline (i.p.) + saline (i.p.)	Amphetamine (2 mg/kg, i.p.)
Subchronic amphetamine	Saline (i.p.) + amphetamine (2 mg/kg, i.p.)	Amphetamine (2 mg/kg, i.p.)
Influence of bupropion on the effect of subchronic amphetamine	Bupropion (5 mg/kg, i.p.) + amphetamine (2 mg/kg, i.p.)	Amphetamine (2 mg/kg, i.p.)
Acute nicotine	Saline (i.p.) + saline (i.p.)	Nicotine (0.1 mg/kg, s.c.)
Development of cross-tolerance between amphetamine and nicotine	Saline (i.p.) + amphetamine (2 mg/kg, i.p.)	Nicotine (0.1 mg/kg, s.c.)
Influence of bupropion on the development of cross-tolerance between amphetamine and nicotine	Bupropion (5 mg/kg, i.p.) + amphetamine (2 mg/kg, i.p.)	Nicotine (0.1 mg/kg, s.c.)

See Figure 4.

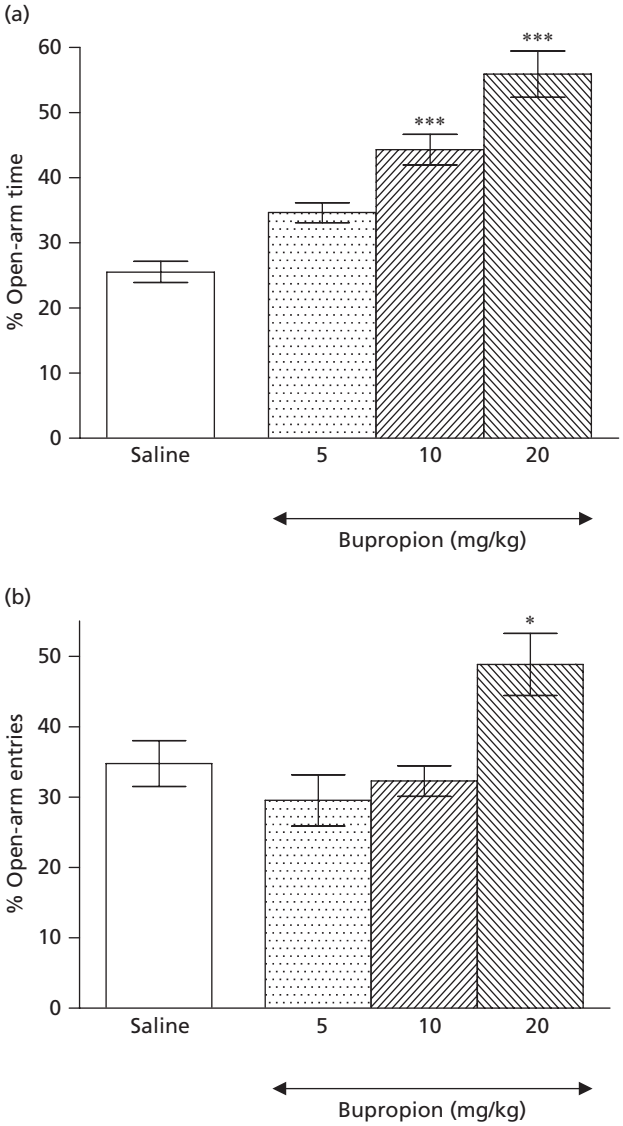

Figure 1 Percentage of time mice spent in open arms during the elevated plus maze test. Values are mean (± SEM). (a) % open-arm time. (b) % open-arm entries. The test took place 30 min after either an acute intraperitoneal injection of bupropion (5, 10 or 20 mg/kg) or saline; $n = 10$; $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with saline control group, Tukey test.

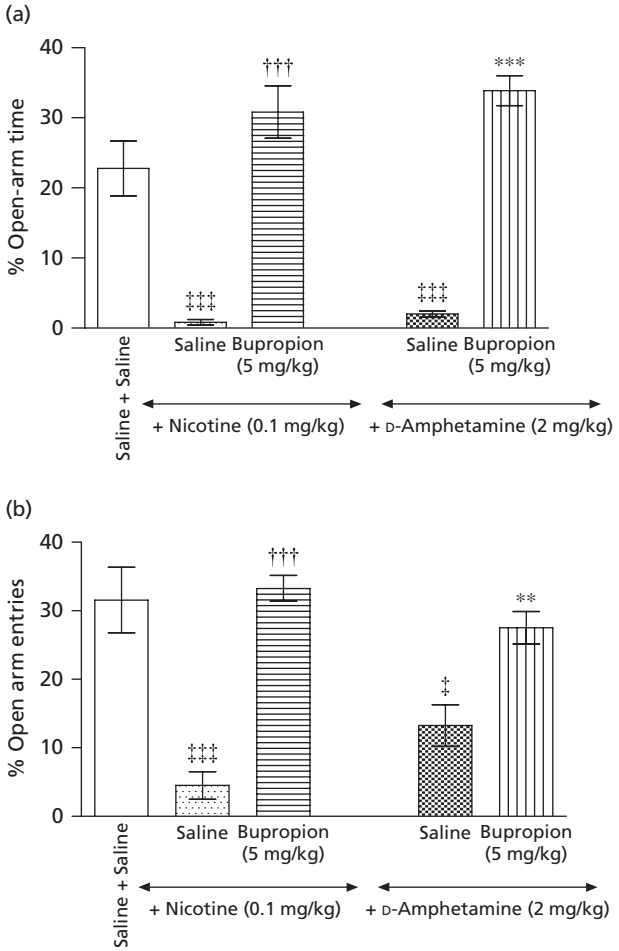

Figure 2 Influence of bupropion on the anxiogenic effect of nicotine or D-amphetamine in mice. Bupropion (5 mg/kg) or saline was administered 15 min before an acute nicotine (0.1 mg/kg, s.c.), D-amphetamine (2 mg/kg, i.p.) or saline injection, and tested 30 min later in the elevated plus maze test. (a) % open-arm time. (b) % open-arm entries. $n = 8$–9; $^{‡}P < 0.05$ and $^{‡‡‡}P < 0.001$ compared with saline control group; $^{†††}P < 0.001$ compared with saline-pretreated and nicotine-tested group; $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with saline-pretreated and amphetamine-treated group, Tukey test.

Influence of bupropion on the acute effect of nicotine and D-amphetamine

Figure 2 also shows the influence of bupropion pretreatment on acute nicotine- and D-amphetamine-induced changes in the behavioural performance of mice in the elevated plus maze test (analysis of variance on the percentage of time spent in open arms: $F_{4,39} = 34.091$, $P < 0.0001$; analysis of variance on the percentage of open-arm entries: $F_{4,39} = 15.591$, $P < 0.0001$). The post-hoc Tukey test indicated that pretreatment with bupropion (5 mg/kg, i.p.) significantly reversed the anxiogenic-like effect of acute nicotine (0.1 mg/kg) or D-amphetamine (2 mg/kg), revealed as the increase in the percentage of time spent in open arms ($P < 0.001$), and the increase in the percentage of open-arm entries ($P < 0.001$ for nicotine, $P < 0.01$ for D-amphetamine), as compared with nicotine- or D-amphetamine-pretreated control group.

Effects of subchronic injections of nicotine and D-amphetamine

Animals were tested 30 min after the seventh injection of nicotine (0.1 mg/kg). The animals showed a significant increase in the time spent in open arms ($P < 0.001$; Figure 3), as well as an increased number of entries to these arms ($P < 0.05$; Figure 3), as compared with the acute nicotine group. This suggested that tolerance developed to the anxiogenic effect of nicotine. Similarly, mice tested 30 min after the ninth injection of D-amphetamine (2 mg/kg) showed increased time spent in open arms ($P < 0.001$; Figure 4), as well as an increased number of entries to these arms ($P < 0.05$;

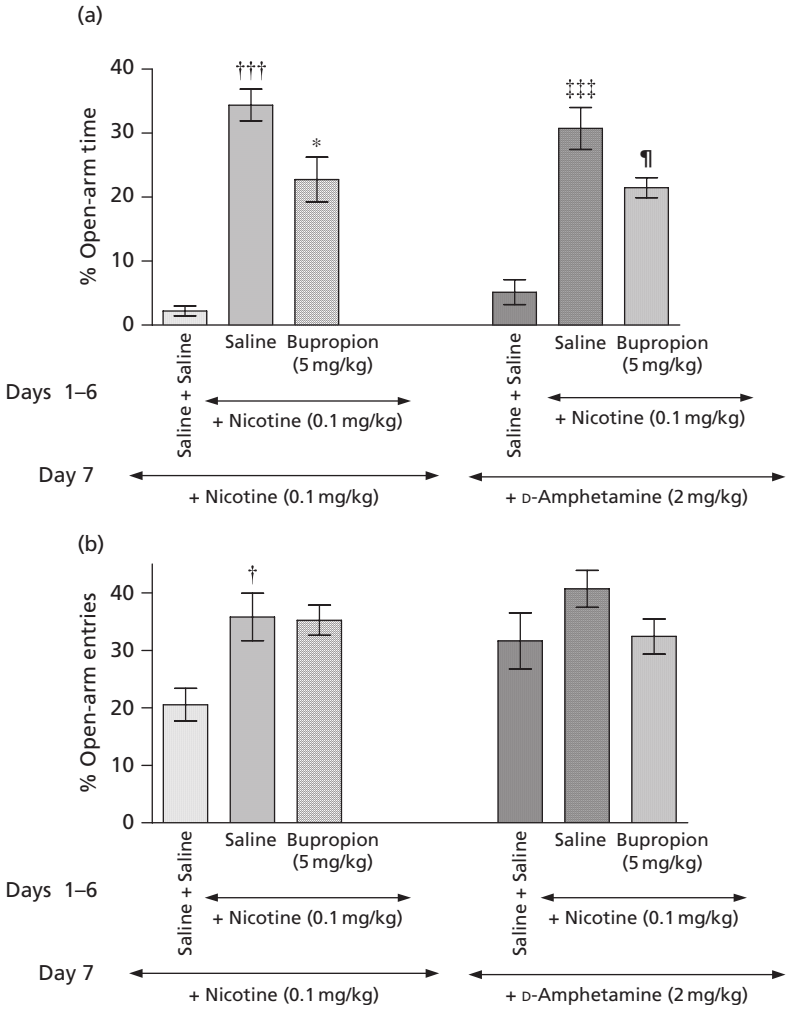

Figure 3 Influence of bupropion on the development of tolerance to nicotine and cross-tolerance between nicotine and D-amphetamine in mice. Mice were subjected to the elevated plus maze test to observe any anxiogenic effect. The dose of nicotine was subchronic. Bupropion (5 mg/kg, i.p.) or saline was administered for six days, 15 min before each daily nicotine or saline injection, and tested on day seven, 30 min after nicotine (0.1 mg/kg, s.c.) or D-amphetamine (2 mg/kg, i.p.) challenge injection (see also Table 1). (a) % open-arm time. (b) % open-arm entries. $n = 8–10$; $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ compared with saline-treated and nicotine-challenged group; $^{*}P < 0.05$ compared with nicotine-treated and nicotine-challenged group; $^{\ddagger\ddagger\ddagger}P < 0.001$ compared with saline-treated and amphetamine-challenged group; $^{\P}P < 0.05$ compared with nicotine-treated and amphetamine-challenged group, Tukey test.

Figure 4), compared with the acute D-amphetamine group, suggesting that tolerance developed to the anxiogenic effect of D-amphetamine.

Influence of bupropion on the subchronic effect of nicotine and D-amphetamine including crossover effects

Pretreatment with bupropion before every daily injection of subchronic nicotine (0.1 mg/kg) or D-amphetamine (2 mg/kg) also influenced the anxiety-related response and the development of tolerance (analysis of variance on the percentage of time spent in open arms: $F_{5,46} = 35.402$, $P < 0.0001$; analysis of variance on the percentage of open-arm entries: $F_{5,46} = 7.892$, $P < 0.0001$). Actually, bupropion (5 mg/kg) abolished the anxiolytic-like effect of subchronic nicotine or D-amphetamine, revealed as decreased time spent in open arms ($P < 0.05$) and decreased number of open-arm entries ($P < 0.001$, for amphetamine-treated group) (Figures 3 and 4). Finally, the nicotine challenge dose (0.1 mg/kg, s.c.) also resulted in an anxiolytic effect in amphetamine-treated mice ($P < 0.001$ for the percentage of time spent in open arms, Figure 3). Similarly, D-amphetamine challenge provoked an anxiolytic action in nicotine-pretreated mice ($P < 0.001$ for the percentage of time spent in the open arms, $P < 0.01$ for the percentage of open-arm entries, Figure 4). These effects suggested the development of cross-tolerance between D-amphetamine and nicotine to their anxiogenic action under the experimental conditions. Additionally, pretreatment with bupropion before every daily injection of subchronic nicotine (0.1 mg/kg, 6 days) or D-amphetamine (2 mg/kg, 8 days) influenced the anxiety-related behaviour in the response to a

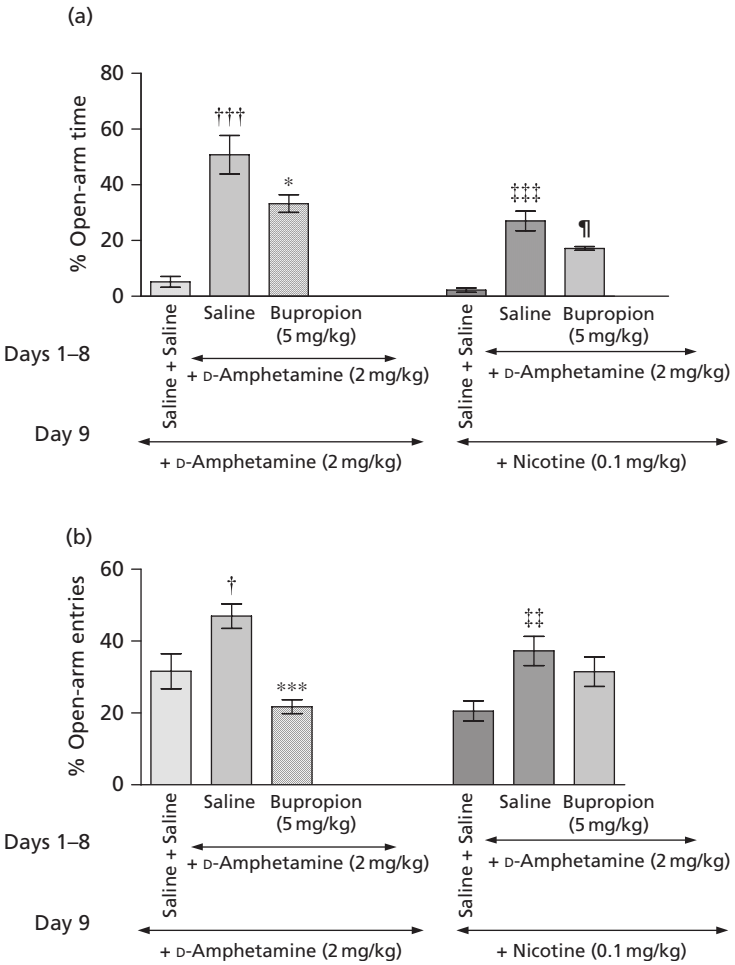

Figure 4 Influence of bupropion on the development of tolerance to amphetamine and cross-tolerance between D-amphetamine and nicotine in mice. Mice were subjected to the elevated plus maze test to observe any anxiogenic action. The dose of D-amphetamine was subchronic. Bupropion (5 mg/kg, i.p.) or saline were administered for eight days, 15 min before each daily D-amphetamine or saline injection, and tested on day nine, 30 min after D-amphetamine (2 mg/kg, i.p.) or nicotine (0.1 mg/kg, s.c.) challenge injection (see also Table 2). (a) % open-arm time. (b) % open-arm entries. $n = 8–9$; $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ compared with saline-treated and amphetamine-challenged group; $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with amphetamine-treated and amphetamine-challenged group; $^{\ddagger\ddagger}P < 0.01$ and $^{\ddagger\ddagger\ddagger}P < 0.001$ compared with saline-treated and nicotine-challenged group; $^{\P}P < 0.05$ compared with amphetamine-treated and nicotine-challenged group, Tukey test.

D-amphetamine or nicotine challenge, i.e. the development of cross-tolerance (analysis of variance on the percentage of time spent in the open arms: $F_{5,46} = 25.586$, $P < 0.0001$; analysis of variance on the percentage of open-arm entries: $F_{5,46} = 3.436$, $P = 0.01$) (Figures 3 and 4). Actually, bupropion (5 mg/kg) abolished the anxiolytic-like effect in mice subjected to subchronic nicotine and challenged with D-amphetamine as well as in mice subjected to chronic D-amphetamine and challenged with nicotine, revealed as the decrease in the percentage of time spent in open arms ($P < 0.05$; Figures 3 and 4, respectively).

Locomotor effects of drugs tested measured in the elevated plus maze paradigm

Moreover, all compounds tested, alone or in combination, given acutely or repeatedly at the doses used, did not provoke any changes in number of closed-arm entries in the elevated plus maze test (Tables 3–5). Thus, except for two groups (bupropion

Table 3 Number of closed-arm entries in the elevated plus maze test in mice

Treatment	Closed-arm entries
Saline control group	13.1 ± 0.95
Bupropion 5 mg/kg	13.3 ± 2.36
Bupropion 10 mg/kg	14.3 ± 0.71
Bupropion 20 mg/kg	11.6 ± 0.85
Saline + saline	14.55 ± 1.22
Saline + nicotine 0.1 mg/kg	12.33 ± 2.35
Bupropion 5 mg/kg + nicotine 0.1 mg/kg	11.62 ± 2.02
Saline + D-amphetamine 2 mg/kg	15.44 ± 1.80
Bupropion 5 mg/kg + D-amphetamine 2 mg/kg	25.37 ± 2.96*

Values are mean ± SEM. Bupropion (5 mg/kg, i.p.) or saline was administered 15 min before acute nicotine (0.1 mg/kg, s.c.), D-amphetamine (2 mg/kg, i.p.) or saline injection. Mice were tested 30 min later in the elevated plus maze test. $n = 8–9$; $^{*}P < 0.05$ compared with saline-pretreated and amphetamine-tested group, Tukey test.

Table 4 Number of closed-arm entries in the elevated plus maze test in mice

Treatment day 1–6	Treatment day 7	Closed-arm entries
Saline + saline	Nicotine 0.1 mg/kg	10.25 ± 0.79
Saline + nicotine 0.1 mg/kg	Nicotine 0.1 mg/kg	12.60 ± 1.28
Bupropion 5 mg/kg + nicotine 0.1 mg/kg	Nicotine 0.1 mg/kg	12.12 ± 0.66
Saline + saline	D-Amphetamine 2 mg/kg	11.87 ± 3.04
Saline + nicotine 0.1 mg/kg	D-Amphetamine 2 mg/kg	10.23 ± 0.52
Bupropion 5 mg/kg + nicotine 0.1 mg/kg	D-Amphetamine 2 mg/kg	11.55 ± 1.82

Values are mean ± SEM. Bupropion (5 mg/kg, i.p.) or saline was administered for six days, 15 min before each daily nicotine (0.1 mg/kg, s.c.) or saline injection. Mice were tested on the seventh day, 30 min after nicotine (0.1 mg/kg, s.c.) or D-amphetamine (2 mg/kg, i.p.) challenge injection. $n = 8–10$.

Table 5 Number of closed-arm entries in the elevated plus maze test in mice

Treatment day 1–8	Treatment day 9	Closed-arm entries
Bupropion 5 mg/kg + saline	Bupropion 5 mg/kg	11.67 ± 1.50
Saline + saline	D-Amphetamine 2 mg/kg	11.87 ± 3.04
Saline + D-amphetamine 2 mg/kg	D-Amphetamine 2 mg/kg	13.23 ± 2.40
Bupropion 5 mg/kg + D-amphetamine 2 mg/kg	D-Amphetamine 2 mg/kg	20.50 ± 1.58*
Saline + saline	Nicotine 0.1 mg/kg	10.25 ± 0.79
Saline + D-amphetamine2 mg/kg	Nicotine 0.1 mg/kg	11.37 ± 1.97
Bupropion 5 mg/kg + D-amphetamine 2 mg/kg	Nicotine 0.1 mg/kg	12.37 ± 0.96

Values are mean ± SEM. Bupropion (5 mg/kg, i.p.) or saline was administered for eight days, 15 min before each daily amphetamine (2 mg/kg, i.p.), or saline injection. Mice were tested on the ninth day, 30 min after D-amphetamine (2 mg/kg, i.p.) or nicotine (0.1 mg/kg, s.c.) challenge injection. $n = 8–9$; $^*P < 0.05$ compared with D-amphetamine-treated and D-amphetamine-challenged group, Tukey test.

5 mg/kg + acute D-amphetamine 2 mg/kg (Table 3) and bupropion 5 mg/kg + D-amphetamine 2 mg/kg in D-amphetamine-pretreated mice (Table 5)) these substances did not change locomotor activity of animals in this paradigm.

Discussion

The primary objective of this study was to explore the phenomenon of tolerance and full cross-tolerance to the anxiogenic responses between nicotine and D-amphetamine, two psychostimulant drugs with a different primary mechanism of action at the molecular level. Based on the existing results and considering that anxiety and emotional disorders are important factors for the establishment of psychostimulant addiction, it seemed of interest to evaluate the anxiety-related effects of acute and subchronic administration of nicotine and D-amphetamine alone or in combination with the antismoking agent bupropion.[5,23]

Nicotine and amphetamine act as reinforcers in humans and animals but, paradoxically, after an acute administration they also have anxiogenic effects in animal tests, and can increase anxiety in humans. As already stated, in animal models of anxiety the administration of nicotine and D-amphetamine may have different effects, being either anxiolytic, anxiogenic, or producing no effects.[8,11,21] Increased anxiety has been reported also on withdrawal from psychostimulant drugs in animals and in smokers.[23,6] Our results have confirmed recent data showing that in the elevated plus maze test, a single injection of a low dose of nicotine and D-amphetamine had a significant anxiogenic effect in mice.[21] Moreover, tolerance developed rapidly to this effect when these anxiogenic doses of both psychostimulant drugs were administered repeatedly, with a full cross-tolerance to these effects. Rapid development of tolerance to the anxiogenic effect of amphetamine or nicotine, when a behavioural response was seen in the opposite direction of the acute drug effect, was likely to be due to the pharmacodynamic mechanisms that involved the progressive recruitment of processes opposing the acute effect of the drug.[8] Mechanisms of the development of tolerance to psychostimulant drugs are not well established and can be explained by changes in receptor numbers or in second messenger cascades.[24] It was possible, therefore, that pretreatment with nicotine exerted complex effects on the response to systemic D-amphetamine (and vice-versa), and may have been derived from alterations of a number of neurotransmitter systems. In our study, the fact that full crossover effects developed between nicotine and D-amphetamine suggested that their behavioural anxiety-related effects observed in the elevated plus maze were controlled by the same neural mechanisms. As such, our data also revealed that bupropion, at an acute non-active dose, decreased anxiogenic effects of both nicotine and D-amphetamine as well as the development of tolerance and cross-tolerance to anxiogenic effects of these drugs after their subchronic administration as measured in the elevated plus maze test in mice.

Nicotine and amphetamine are often abused in combination. The two drugs can facilitate each other's intake, and produce similar subjective and physiological effects in humans. Thus, there is considerable evidence indicating commonalities between their behavioural effects.[25] Although the molecular targets underlying the pharmacological responses to nicotine and amphetamine differ considerably

(somatodendric nAChRs and presynaptic dopamine transporter on nerve terminal membranes, respectively), their effects depend upon enhanced neurotransmission at dopaminergic synapses.[16,18] It has been well documented that repeated administration of nicotine or D-amphetamine (and/or cocaine) produced tolerance or sensitization to some of their behavioural (e.g. locomotion) and neurochemical effects, depending upon the paradigm used and the interval between injections.[26,27] Moreover, prior exposure to one drug was likely to alter the behavioural effects of the other, and cross-tolerance or cross-sensitization often occurred after their repeated treatment.[21,28–30] It is of interest to note that a nicotinic receptor antagonist, mecamylamine, prevented the acquisition of long-term behavioural sensitization of amphetamine.[26] Mecamylamine also abolished the development of neurochemical sensitization of mesolimbic neurons, i.e. long-term hyperactivity of dopamine neurons in the nucleus accumbens in nicotine-, amphetamine- or cocaine-pretreated rats.[26] Thus, nicotinic receptor activation may be a common neural mechanism underlying the development of psychostimulant-induced sensitization and/or tolerance, i.e. the processes of synaptic plasticity.

The major finding of this study was a blockade of acute or subchronic effects, including cross-actions of both psychostimulants by bupropion, an antidepressant drug widely used to facilitate smoking cessation in humans. To our knowledge, this kind of study has not been elucidated yet. At present, bupropion represents an alternative to the conventional nicotine replacement therapies of nicotine addiction.[31] In the context of dependence and nicotine reward, acute bupropion pretreatment has been reported to produce a biphasic dose–response curve, with low doses increasing intravenous nicotine self-administration, and high doses decreasing the number of nicotine infusions self-administered in rats.[32,33] On the contrary, Shoaib *et al*.[34] have reported that chronic pretreatment with low doses of bupropion increased nicotine self-administration in rats. Furthermore, bupropion has been reported to reduce somatic signs of nicotine withdrawal in rats including gasp, abdominal constriction, teeth chatters, chews, shakes, tremors and ptosis.[2,3] Interactions of bupropion with other psychostimulants such as D-amphetamine, especially their influence on anxiety-related processes, have not been commonly reported. The mechanism of action of bupropion has not been fully understood, as at least two different targets have been proposed. The preclinical and clinical literature demonstrated that bupropion nonselectively inhibited the reuptake of dopamine and noradrenaline by inhibition of the dopamine and noradrenaline transporters, as well as having nAChR antagonist activity, and each of these effects may have contributed to its efficacy as both an antidepressant and tobacco use cessation agent.[1,4] As animal studies, including present data, indicated that bupropion blocked behavioural effects of nicotine, an antagonistic action at the nAChR could explain (in part) a blunting of nicotine's effects, especially that these receptors are also implicated in learning and memory processes, reward, antinociception and anxiety.[20,35] It cannot be excluded that action of bupropion on dopamine or noradrenaline neurons can also play a role. In accordance, bupropion has been shown to activate catecholaminergic neurotransmission, and these systems have been found to directly control aversive-related processing, including anxiogenic effects.[3] In this context, a growing body of evidence suggested that the mesolimbic dopamine pathways originating from the ventral tegmental area (i.e. amygdala, prefrontal cortex and nucleus accumbens) play an important role in the addictive properties of illicit drugs as well as in different aspects of anxiety, aversive reinforcement and fear conditioning.[36] Slemmer *et al.*[1] claimed that the ability of bupropion to antagonize the unconditioned behavioural effects of nicotine (e.g. antinociception or hypoactivity) were mediated by nAChRs, while Malin *et al.*[3] suggested that catecholaminergic mechanisms may have accounted for the ability of bupropion pretreatment to antagonize the somatic signs of nicotine withdrawal in rodents. It has been commonly believed that one of the primary mechanisms of action of bupropion as a smoking cessation drug involves the dopaminergic system.[37] What is more, bupropion dose-dependently inhibited nicotine-evoked [H^3]dopamine overflow from rat striatal and hippocampal slices *in vitro*.[38] These data may suggest that bupropion could help smokers quit the habit by elevating extracellular dopamine level, thus alleviating withdrawal symptoms.

Interactions of bupropion with D-amphetamine are even more complex. Like D-amphetamine, bupropion stimulated locomotor activity, produced stereotypies and reduced eating.[39,40] Thus, both drugs affected dopamine transmission differently. Bupropion and its metabolites displayed an inhibition of dopamine uptake and, in contrast to D-amphetamine, were devoid of dopamine releasing effects.[41] Hence its modest inhibition of dopamine and/or noradrenaline reuptake could be considered to be of major importance in attenuating D-amphetamine anxiety-related effects, also observed in this study. In accordance with data showing that bupropion could diminish some effects of psychostimulants, a recent clinical study has revealed that this antidepressant, in combination with behavioural therapy, was effective for increasing the time of abstinence in metamphetamine abuse.[42]

Conclusions

This investigation was designed to examine further the anxiety-related behavioural consequences of acute and subchronic D-amphetamine or nicotine in the mouse elevated plus maze test of anxiety. Firstly, it has been confirmed that after chronic administration, tolerance and especially cross-tolerance developed to the anxiogenic action of D-amphetamine and nicotine, which could suggest that a similar mechanism contributed to the expression of these effects. Secondly, consistent with some results pointing out the involvement of bupropion in the response to psychostimulant drugs, we have established that administration of this antidepressant before every acute or chronic injection of nicotine or D-amphetamine attenuated their anxiety-related effects as well as the development of cross-tolerance to these effects in mice. Taking all these results together, it seemed plausible to speculate about an influence of bupropion in the adaptive changes underlying anxiety behaviour which could be considered one of the major signs in patients after abrupt

withdrawal of psychostimulant drugs. This is especially important as smoking cigarettes may be an increased risk for amphetamine abuse. Identifying the mechanism involved in anxiety-related responses could lead to therapies of nicotine and/or amphetamine addiction. Thus, more research is necessary to improve understanding of the mechanisms behind the efficacy of bupropion as a smoking cessation agent, which can be effective also in polydrug abuse.

Recently, the efficacy of bupropion has been compared with a new non-nicotine smoking-cessation agent varenicline, a partial agonist at alpha4beta2 nAChRs.[43] In a randomized double-blind parallel-group study, sustained-release bupropion was shown to be less efficacious than varenicline. Since varenicline and bupropion appear to have somewhat different mechanisms of action, further improvements in abstinence rates could probably be realized by employing these drugs in combination.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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