REVIEW

Interaction of nicotinic receptors with bupropion: Structural, functional, and pre-clinical perspectives

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Besides the antidepressant activity of bupropion (BP), preclinical studies in rodents provide evidence that this compound and its hydroxyl metabolites can attenuate nicotine withdrawal, reversing both the physical and negative affective aspects of nicotine abstinence. Co-interactions of BP with nicotine or other psychostimulants influence decrease anxiety- and cognitive- related processes. BP also attenuates the reinstatement of nicotine-induced conditioned place preference in rats caused by a priming dose of nicotine, morphine, cannabinoids, or ethanol. Therefore, BP can offer an interesting approach to the prevention of relapse in humans. There is emerging evidence that BP inhibits, in the low to intermediate micromolar range, various nicotinic acetylcholine receptors (AChRs) expressed in different neuronal pathways. The BP selectivity for different AChRs follows the sequence: α3- > α4- ~ α1- > α7-containing AChRs. This receptor blockade may contribute to its dual therapeutic activity as either an antidepressant or anti-nicotinic drug. Regarding the structural aspects, two distinct binding sites for [^{125}I]SADU-3-72, a photosensitive derivative of BP, were identified in the *Torpedo* AChR. A binding site is located within the ion channel, which coincides with the molecular docking results, whereas a second site is found near the extracellular end of α1-M1 when the receptor is in the desensitized state. Interestingly, BP greatly reduces the potentiating action elicited by Zn^{2+} on different non-α7 AChRs, and vice versa this cation increases the inhibitory strength of BP. This contrasting behavior supports the concept that the binding sites for BP and $Zn^{2+}$are located at different domains. The understanding of the BP activity, alone or in combination with other drugs (e.g., nicotine), at the molecular and behavioral levels, may improve the knowledge of the underlying mechanisms of action. This knowledge is essential for the development of novel BP (or other antidepressant) derivatives with improved clinical profiles for the treatment of depression and psychostimulant-related addictions.

***Abbreviations*:** BP, bupropion; CNS, central nervous system; AChR, nicotinic acetylcholine receptor; VTA, ventral tegmental area; EPM, elevated plus maze; mEPM, modified EPM; TL, transfer latency; CPP, conditioned place preference; GABA, γ-aminobutyric acid; NCA, noncompetitive antagonist; NE, noradrenaline; DA, dopamine; IC_{50}, ligand concentration that inhibits 50% binding; K_i, inhibition constant; SADU-3-72, 2-(N-tert-butylamino)-3'-iodo-4'-azidopropiophenone; [^{3}H]TCP, [piperidyl-3,4-3H(N)]-(N-(1-(2-thienyl)cyclohexyl)-3,4-piperidine); α-BTx, α-bungarotoxin; CCh, carbamylcholine; PCP, phencyclidine.

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Introduction

Bupropion (BP) (Figure 1) is a Food and Drug Administration (FDA)-approved medication for the treatment of depression and is recommended as a first aid for smoking cessation [1, 2]. The clinical efficacy of BP can be attributed to two combined properties: inhibition of neurotransmitter reuptake such as noradrenaline (NE) and dopamine (DA) and eliciting stimulatory effects in the central nervous system (CNS) similar to that produced by nicotine, and noncompetitive inhibition of several nicotinic acetylcholine receptors (AChRs) [1-3]. AChRs are members of the Cys-loop ligand-gated ion channel superfamily that includes type 3 5-hydroxytryptamine (serotonin), type A and C γ-aminobutyric acid (GABA), and glycine receptors found in vertebrates, and additional ligand-gated receptors found in invertebrates [2, 4-7].

In addition to the very well characterized activity of BP as an antidepressant [8], this drug displays an extensive spectrum of behavioral effects. For instance, BP causes dose-dependent sniffing [9], hypothermia [10], anorexia [11], and convulsant activity [12]. BP also improves performance in some learning tasks [13, 14]. Emotional effects of BP have also been observed in social interactions in mice [15]. Moreover, BP induces locomotor stimulation, although this activity is mild [16-18]. BP has also been found to decrease not only nicotine but also the addictive and craving effects elicited by methamphetamine in humans [19], and by cocaine in rodents [20], as well as morphine-induced tolerance and dependence in rodents [21].

This review focuses on the pharmacological, functional, and behavioral activity of BP as well as the components of its binding sites in the AChR.

Antidepressant activity of BP

Depression is a common mental disorder characterized by the presence of at least five of the following symptoms [22]: depressed mood, decrease or increase of appetite correlated with respective weight gain or weight loss, psychomotor agitation or retardation, diminished concentration and interest or pleasure in almost all activities (i.e., anhedonia), insomnia or hypersomnia, loss of energy, feelings of worthlessness or/and excessive or inappropriate guilt, and recurrent thoughts of death and/or suicidal ideation.

BP has been used as an antidepressant (Wellbutrin®)[8] for the treatment of clinical depression as well as atypical depression that is associated with interpersonal deficits such as rejection sensitivity and social avoidance [23]. BP is also the only antidepressant that has high efficacy to prevent depressive relapse for seasonal affective disorders [24].

Preclinical studies using the forced swimming and tail suspension tests indicate that BP increases the mobility time of rodents [25-27]. Previous results indicate that β4-containing AChRs play an important role in the chronic antidepressant activity elicited by BP [27]. In particular, an antidepressant effect was seen only in wild-type (β4+/+) female mice but not in knockout (β4-/-) mice after the chronic treatment with BP (Figure 2). A potential explanation for the observed results is that the removal of the β4 subunit from the mouse prevents the formation of β4-containing AChRs. The habenulo-interpeduncular pathway expresses α3β4 and α3β3β4 AChRs [28], and modulates directly and indirectly the mesocorticolimbic dopaminergic circuitry [29]. In this regard, the absence of β4-containing AChRs makes the cholinergic pathway dysfunctional, and thereby enhancing the mesocorticolimbic dopaminergic transmission that is fully functional since the expression of β4-containing AChRs is less important or other AChR subtypes are involved instead. Our results also show that the residual antidepressant effect of BP persisted only in male β4+/+ mice after one week withdrawal and the chronic antidepressant activity is observed only in female β4+/+ mice (Figure 2), clearly indicating that the antidepressant effect of BP is gender-specific. Although some evidence indicates that there is no sex differences in the antidepressant activity mediated by BP [30], this drug is more effective in women compared to men when it is used in the pharmacotherapy of smoking cessation. These gender differences could be due to variations in pharmacokinetics and metabolism of BP [31]. An alternative interpretation for the observed results between female and male β4+/+ mice is that male β4+/+ mice are less sensitive to the anhedonic effects following BP withdrawal compared to female β4+/+ mice [27].

In addition to its activity on the habenulo-interpeduncular pathway, BP inhibits AChRs expressed in the mesocorticolimbic system. In the ventral tegmental area (VTA), α7 (i.e., homomeric) as well as non-α7 (i.e., heteromeric) AChRs are expressed on dopaminergic, GABAergic, and glutamatergic neurons [32-35]. In particular, BP blocks presynaptic α4β2-containing AChRs on GABAergic neurons which diminishes tonic inhibition of VTA neurons [36]. This activity enhances DA release, which may contribute to its antidepressant action [37]. The activation of presynaptic α7 AChRs enhances the glutamatergic transmission onto DA neurons [33], promoting long-term potentiation and synaptic plasticity in the VTA [36]. Synaptic plasticity in the VTA may be induced after smoking a single cigarette [36, 38] and most likely underlies the persistent effects of nicotine. Thus, the BP-induced α7 AChR inhibition decreases DA release in the nucleus accumbens and prefrontal cortex as well as the concomitant long-term potentiation [38], finally decreasing nicotine craving.

In the hippocampus, the increase of ACh signaling *via* physostigmine-induced AChE inhibition promotes anxiety

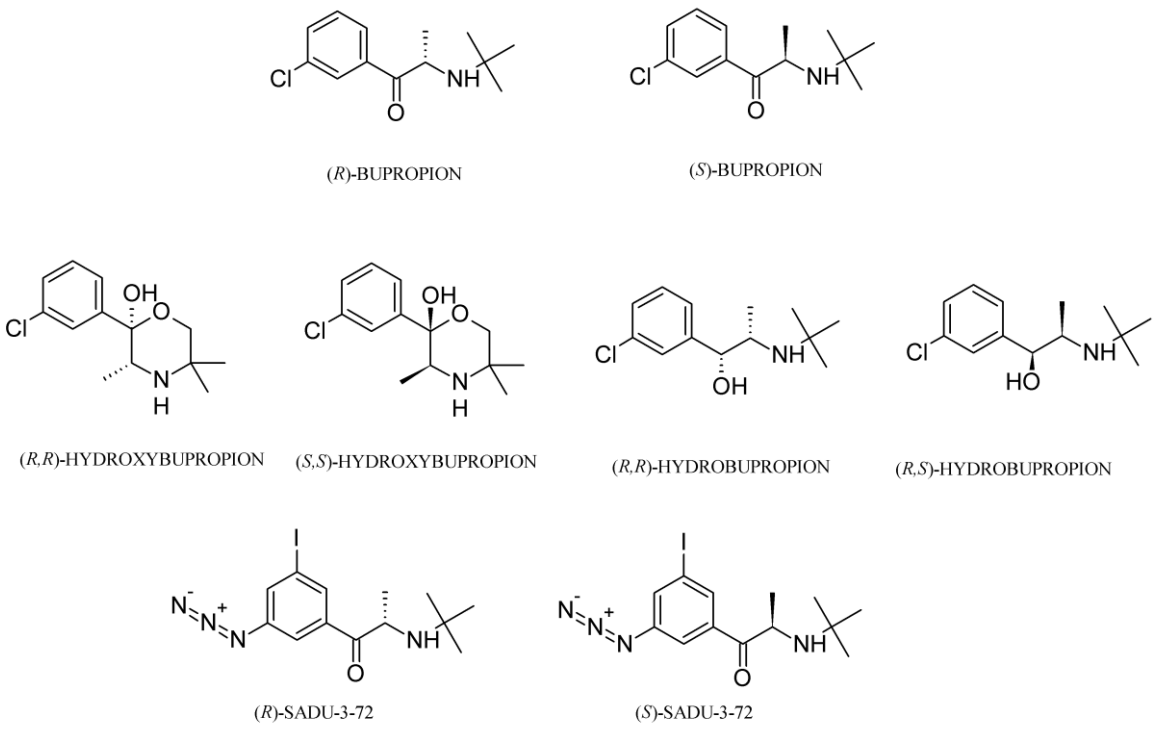

Figure 1. Chemical structures of the BP enantiomers, metabolites, and derivatives. In particular, (*R,R*)-hydroxybupropion, (*S,S*)-hydroxybupropion, (*R,R*)-hydrobupropion, and (*R,S*)-hydrobupropion, as well as its photoreactive enantiomers, (*R*)- and (*S*)-SADU-3-72. The iodine atom and the photoreactive azide group (possessing negative and positive charges) are located at positions 3' and 4', respectively, on the aromatic ring of SADU-3-72.

and depression, whereas the inhibition of the cholinergic transmission by nicotinic antagonists induces antidepressant-like activity [39]. Experiments using hippocampal neurons demonstrated that BP inhibits $\alpha4\beta2$-, $\alpha3\beta4$- [40], and $\alpha7$-containing AChRs (submitted manuscript) with inhibitory potencies in the same concentration range as that obtained by *in vitro* studies (Table 1). Moreover, BP decreases nicotine-induced up-regulation of $\alpha4\beta2$ and $\alpha3\beta4$ AChRs expressed in hippocampal neurons [40]. Since AChR up-regulation is an important stage in the process of nicotine addiction [41], the observed activity of BP could be part of the therapeutic effects mediated by this antidepressant.

The most accepted mechanism of action for the BP antidepressant activity is that this drug inhibits the catecholamine reuptake in presynaptic neurons, modulating the concentrations of the neurotransmitters DA and NE in the synaptic cleft [1, 2]. Since the BP affinity for these neurotransmitter transporters is only moderate [1], the combined inhibition of AChRs and neurotransmitter transporters might account for its clinical efficacy. In particular, the antidepressant activity of BP is mediated by its inhibitory actions on α4β2- and α7-containing AChRs expressed in the VTA and hippocampus, and α3β4-containing AChRs expressed in the habenulo-intrerpeduncular pathway [1, 2].

Activity of BP for smoking cessation

The primary component of tobacco is nicotine, the responsible for the development of addiction. Nicotine dependence is a very complex dysfunction of the CNS, characterized by obsessive drug-craving (defined as an excessive wish for a specific substance or experience), drug-seeking, and drug-taking despite the fact of serious health and life risks. Upon cessation of tobacco smoking or decrease in intake, nicotine withdrawal can occur. The physical and affective symptoms of nicotine withdrawal in humans, include depression, anxiety, headaches, feelings of restlessness or frustration, increased appetite, and difficulty in concentration [42].

In the context of nicotine dependence, BP is a current alternative medicine to the conventional nicotine replacement therapy [1, 8]. There are many reports focused on the involvement of BP in the behavioral effects induced by nicotine. Preclinical evidences indicated that BP and its hydroxyl metabolites diminish nicotine withdrawal in rodents, reversing both the physical and negative affective syndromes of nicotine abstinence. Although BP is considered

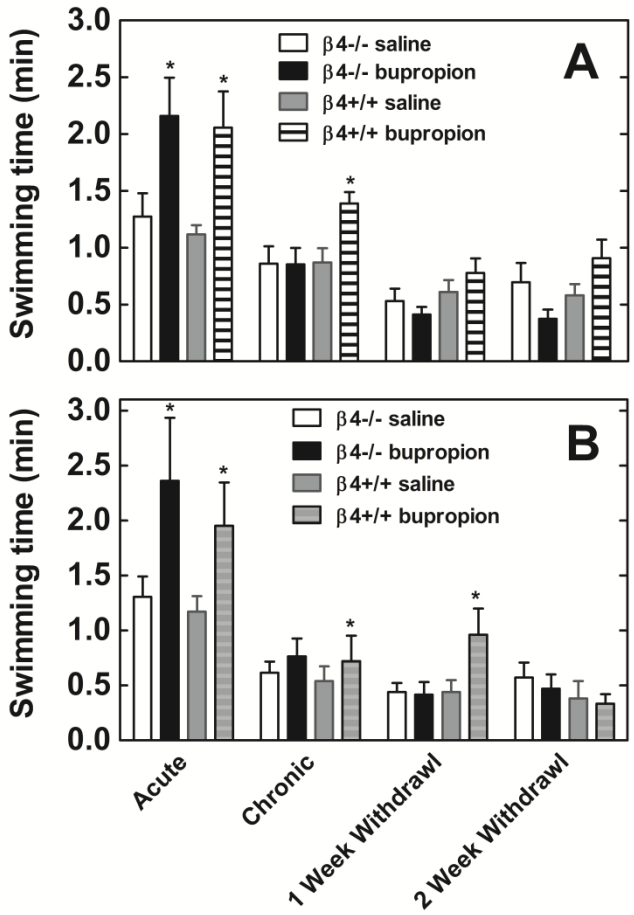

Figure 2. The antidepressant activities mediated by BP in (A) female and (B) male mice determined by FSTs (Reprinted with permission [27]). BP acute antidepressant effect was determined 1 h after the injection, the chronic antidepressant activity of BP was measured after two weeks of daily treatment, and the residual effects of BP was determined after one and two weeks of cessation of BP administration in wild-type (β4+/+) and knockout (β4-/-) mice. *Statistically significant difference from the respective saline-treated control group ($p<0.05$).

a mild psychomotor stimulant, it can attenuate somatic signs of nicotine withdrawal in animal models, including abdominal constriction gasp, writhing, teeth chattering, chewing, tremors, shakes, and ptosis [43-45]. Moreover, the influences of BP on learning and memory impairment that occur during nicotine withdrawal have also been investigated [8, 46-48]. The results revealed that this drug reverses nicotine withdrawal-associated deficiencies in contextual fear conditioning or anxiety-like effects of nicotine withdrawal in mice. In addition, BP reduces nicotine-conditioned taste aversion [14] as well as nicotine-induced antinociception [3]. In the context of dependence and nicotine reward, BP attenuates nicotine-induced unconditioned effects, sharing or enhancing discriminative stimulus properties of nicotine [49], and mediates nicotine self-administration in rats in a complex manner, increasing intravenous nicotine self-administration at low doses, or reducing the number of nicotine self-administered infusions at high doses [50, 51]. On the contrary, Shoaib and co-workers [52] have shown that the chronic pretreatment with low doses of BP increases nicotine self-administration in rodents. Furthermore, BP attenuates nicotine chronic tolerance in mice [53].

Taking into account the available treatment for nicotine dependence, BP and nicotine replacement therapy provide the best clinical outcomes [54]. Although BP has been found to be the single most effective treatment for cigarette smoking in randomized, controlled trials, its combination with nicotine (e.g., patch, gum, or nasal spray) improves the success rate for smoking cessation by easing withdrawal symptoms.

Considering these results and the possible mechanisms of action mentioned above, we can speculate that the clinical effectiveness of BP in the treatment of patients who are motivated in quitting smoking is based on the nicotine-like effects produced by its low doses as well as on the noncompetitive antagonism of AChRs, finally serving as a suitable substitute for nicotine-induced stimulation.

Effect of BP on anxiety

Anxiety is often described as "a psychological, physiological, and behavioral state induced in animals and humans by a threat to well-being or survival, either actual or potential" [55]. This state is characterized by increased autonomic and neuroendocrine activation as well as specific behaviors which can facilitate coping with an unexpected or adverse situation. Anxiety seems to be a key factor, in humans and rodents, for the establishment and maintenance of physical dependence and many drugs withdrawal syndrome of abuse, such as nicotine, amphetamine, alcohol, cocaine, marijuana, and ecstasy. Nicotine has anxiolytic effects but also, despite the subjective feelings, it displays higher levels of anxiety-related behaviors in smokers compared to nonsmokers and smokers who quit [56, 57]. The relationship between anxiety disorders and nicotine dependence is complex. Anxiety might increase smoking behavior and the risk for dependence since increased anxiety may be one of the causes to start smoking. In this context, the use of cigarettes can be considered as an anxiolytic self-treatment. Moreover, nicotine addicts may try to relieve the anxiety during nicotine withdrawal increasing the chances of relapse [58]. Psychiatric disorders, including mania, anxiety as well as panic attacks are commonly observed among amphetamine abusers, especially among first time users [59]. Psychostimulants withdrawal has been also related to states of increased dysphoria and anxiety in human addicts and laboratory animals [60].

Table 1. Inhibitory potency of bupropion, its metabolites and its photosensitive derivative at different AChR subtypes.

AChR subtype	Ligand	Method	IC_{50} [a] (μM)	Reference
hα4β2	(±)-BP	$^{86}Rb^+$ efflux	12.0 ± 1.1	[78]
	(*R,R*)-hydroxy-BP	$^{86}Rb^+$ efflux	31.0 ± 1.0	[78]
	(*S,S*)-hydroxy-BP	$^{86}Rb^+$ efflux	3.3 ± 1.1	[78]
hα4β4	(±)-BP	$^{86}Rb^+$ efflux	14.0 ± 1.1	[78]
	(*R,R*)-hydroxy-BP	$^{86}Rb^+$ efflux	41.0 ± 1.0	[78]
	(*S,S*)-hydroxy-BP	$^{86}Rb^+$ efflux	30.0 ± 1.1	[78]
hα3β4	(±)-BP	$^{86}Rb^+$ efflux	1.51 ± 0.32[b]	[96]
	(±)-BP	Voltage-clamp	8.0± 2.6	[3]
		$^{86}Rb^+$ efflux	7	[97]
rα3β4	(*R,R*)-hydro-BP	$^{86}Rb^+$ efflux	14	[97]
	(*R,R*)-hydroxy-BP	$^{86}Rb^+$ Efflu	2	[97]
	(*S,S*)-hydroxy-BP	$^{86}Rb^+$ efflux	18	[97]
rα3β2	(±)-BP	Voltage-clamp	1.3± 0.6	[3]
rα7	(±)-BP	Voltage-clamp	~60	[3]
hα3β4*	(±)-BP	Ca^{2+} influx	0.82±0.10[b]	[98]
		$^{86}Rb^+$ efflux	1.8±1.1	[78]
	(*R,R*)-hydroxy-BP	$^{86}Rb^+$ efflux	6.5±1.0	[78]
	(*S,S*)-hydroxy-BP	$^{86}Rb^+$ efflux	10.0±1.5	[78]
hα1β1γδ		$^{86}Rb^+$ efflux	7.9±1.0	[78]
	(±)-BP	$^{86}Rb^+$ efflux	10.5±1.0	[96]
		Ca^{2+} influx	23.4±4.5	[79]
	(*R,R*)-hydroxy-BP	$^{86}Rb^+$ efflux	7.6±1.0	[78]
	(*S,S*)-hydroxy-BP	$^{86}Rb^+$ efflux	28.0±1.4	[78]
hα1β1εδ	(±)-SADU-3-72	Ca^{2+} influx	1.4±0.2	[80]
	(±)-BP	Ca^{2+} influx	24.4±3.3	[79]
	(±)-SADU-3-72	Ca^{2+} influx	4.1±0.7	[80]
mα1β1εδ	(*R,R*)-hydroxy-BP	$^{86}Rb^+$ efflux	7.6±1.0	[78]
	(*S,S*)-hydroxy-BP	$^{86}Rb^+$ efflux	28.0±1.4	[78]
Tα1β1γδ	(±)-BP	Voltage-clamp	0.34±0.07	[81]

[a] IC_{50} is the required drug concentration to produce 50% inhibition of agonist-activated AChRs.
[b] The native AChR can have additional subunits.
h, human; r, rat; m, mouse; T, *Torpedo*

Although the anxiolytic/anxiogenic activity of BP has been determined in different animal models, the mechanism of action by which this drug induces its therapeutic effects and its combination with nicotine or other psychoactive drugs is still not completely understood. As we previously mentioned, BP may diminish nicotine withdrawal symptoms (including anxiety-related responses), however its effects especially on anxiety is unclear [61, 62]. Co-interactions of BP with other psychostimulants, not only nicotine, especially their involvement in anxiety-related responses, have not been reported yet. In this regard, Biała's laboratory has investigated the influence of BP on anxiety-associated responses as well as on the acute and subchronic anxiety-related actions elicited by nicotine and D-amphetamine, and on the acquisition of full cross-tolerance between nicotine and D-amphetamine [63]. It can be mentioned that a variety of abused drugs have been reported to modulate the expression of anxiety. Along with reward enhancement and cognitive improvement, anxiolysis is one of the main effects underlying nicotine and/or amphetamine dependence. It has been proposed that the negative affective effects (e.g., anxiogenic) related to nicotine or D-amphetamine

Table 2. Binding affinities of BP and (±)-SADU-3-72 for *Torpedo* AChRs in different conformational states.

Radioligand	Ligand	Resting/α-BTx-bound state	Desensitized/agonist-bound state	Reference
		K_i [a] (μM)	K_i [a] (μM)	
[^{3}H]TCP	(±)-BP	5.1 ± 0.3	2.0 ± 0.1	[79]
	(±)-SADU-3-72	0.75 ± 0.04	0.89 ± 0.05	[80]
[^{3}H]Imipramine	(±)-BP	21.4 ± 3.4	11.6 ± 1.3	[79]
[^{125}I]SADU-3-72	(±)-SADU-3-72	0.07 ± 0.07 [b]	0.80 ± 0.08	[81]
	(±)-BP	3.6 ± 0.5 [b]	1.2 ± 0.3	[81]

[a] The inhibition constant (K_i) represents the ligand affinity for the particular radioligand binding site(s).
[b] Experiments performed in the absence of any ligand (i.e., AChRs mainly in the resting state).
h, human; T, *Torpedo*.

withdrawal mediate continued drug use and contribute to the observed high relapse rate [64]. To measure anxiety-associated responses in mice, the elevated plus maze test (EPM) was used. The experimental apparatus consists of a central platform and four equal-sized arms: two open and two closed, located opposite to each other. The experimental maze is made of dark Plexiglas, elevated to a height of 50 cm above the floor and illuminated by a dim light. The paradigm consists of placing the mouse in the central platform facing an enclosed arm and allowing it to freely explore the maze for 5 min, and counting the number of entries into the open and closed arms and the time spent in the open arms. An anxiolytic activity is indicated when the time spent in the open arms or the number of open arm entries is increased, whereas an anxiogenic effect is characterized by a decreased time. We found that acute doses of BP (10 and 20 mg/kg) significantly increase the percentage of time spent on the open arms and the percentage of open arm entries (the highest dose) indicating an anxiolytic effect when compared with saline-treated mice. The lowest dose of BP (5 mg/kg) did not cause any effect in this paradigm.

Moreover, we found that that the pretreatment with a non-active dose of BP before the acute treatment with nicotine- or D-amphetamine, induces changes in the behavioral responses in mice in the EPM. More specifically, BP significantly attenuated the anxiogenic-related effect of nicotine (0.035 mg/kg, free base) or D-amphetamine (2 mg/kg). Additionally, pretreatment with BP before every daily injection of subchronic doses of nicotine (0.035 mg/kg) or D-amphetamine (2 mg/kg) modulated the anxiety-associated responses and the tolerance development and cross-tolerance between these psychostimulants. More specifically, BP (5 mg/kg) reduced the anxiolytic-like effect of subchronic doses of nicotine or D-amphetamine [63].

Taking all these results together, it seems plausible that BP influences the adaptive changes related to anxiety behavior, which could mediate one of the basic signs in patients after abrupt psychostimulant drugs withdrawal. This is especially crucial since persons that smoke cigarettes may have an increased risk for amphetamine abuse [65, 66].

In a different set of experiments, we established that BP can attenuate the rewarding (dependence-producing) nicotine effects and also the cognitive responses related to addiction. This also supports the view that there is a similarity in the molecular mechanisms and the brain regions involved in drug addiction and memory-related processes [67]. Therefore, the acute BP effects on memory processes and its influence on the cognitive responses induced by nicotine or scopolamine, an antimuscarinic drug (i.e., on subtypes M1 and M2, expressed in the basal forebrain region) that produces memory impairment, has been studied [68, 69]. Memory and learning responses were determined using the modified EPM (mEPM) test in mice. Basically, this test was originally developed to assess anxiety in animal models. However, recent study proposes that mEPM may be useful in cognitive processes evaluation in rodents [70, 71]. For the mEPM test, the time that the mice takes to move from the open arm to the enclosed arm is defined as transfer latency (TL) and used as an index of cognitive processes. In this regard, mice were placed individually at the end of the open arm facing away from the central platform. Each group was submitted to the same procedure twice (the interval between the trials was 24 h). During the first trial (pretest), the time that each mouse takes to move from the open arm to either of the enclosed arms is recorded as TL1. For the next trial (retention trial) 24 h later, the test is performed in the same manner as the first trial, and the TL (i.e., TL2) is used as an index of memory and learning processes. The memory enhancement is characterized by a decrease in the time necessary for the mouse to move from the open arm to either of the enclosed arms on the second day relative to the control group, while in cognitive deficits are characterized by increases in these measurements. The mEPM task enables us to evaluate different stages of memory depending on the time of drug treatment. In our experiments, the drugs were injected 30 min before the pretest or immediately after the pretest, and the responses of each compound on both acquisition and consolidation of memory were investigated. Our results

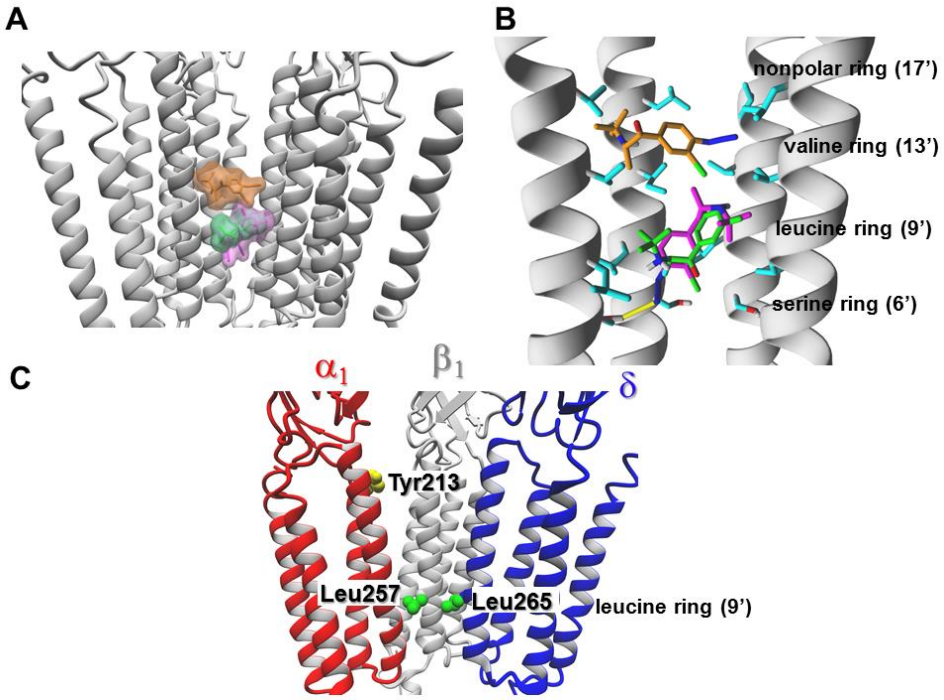

Figure 3. Molecular interaction of (*R*)-BP and (*S*)-SADU-3-72 with the *Torpedo* AChR ion channel. (A) Molecular docking of (*R*)-BP and (*S*)-SADU-3-72 within the *Torpedo* AChR ion channel (modified from [80]). (B) Side view of the overlapping binding sites for (*R*)-BP (green) in the middle of the ion channel and the additional two binding modes for (*S*)-SADU3-72: one closer to the extracellular mouth (orange) and another located in the middle of the ion channel (magenta). (*R*)-BP and (*S*)-SADU-3-72 interact by van der Waals contacts with the valine (position 13'), leucine (position 9'), and serine (position 6') rings. In addition (*S*)-SADU-3-72 forms a hydrogen bond (see yellow arrow) between its azido group and the hydroxyl group of α1-Ser248 (serine ring), and interacts (orange) by van der Waals contacts with the nonpolar (position 17') and valine rings. The δ subunit is removed for clarity. (C) Model of the BP binding sites based on [^{125}I]SADU-3-72 photoaffinity labeling of *Torpedo* AChR (modified from [81]). Residues δ-Leu265 and β1-Leu257 (shown in green), forming the leucine ring, were found photolabeled in the resting state, whereas α1-Tyr213 (shown in orange), located near the extracellular end of α1-M1 was photolabeled in the desensitized state. For clarity two subunits are hidden, the order of explicitly shown is (from left to right) α1 (red), β1 (gray) and δ (blue). Residues involved in ligand interaction are presented either in stick (A and B) or green ball (C) mode, whereas ligands are rendered either in stick/surface (A) or stick (B) mode.

indicate that BP did not significantly alter the TL2 values for both acquisition and consolidation trials at any tested dose (i.e., 10, 20, or 40 mg/kg). However, when experiments where performed combining BP and nicotine, a statistically significant effect caused by BP pretreatment was obtained. Nevertheless, BP (10 or 20 mg/kg) prevented improvements on memory acquisition and memory consolidation elicited by the administration of 0.035 mg/kg nicotine [68, 69]. This effect suggests that BP treatment can diminish not only the rewarding (dependence-producing) effects of nicotine, but also the cognitive effects related to addiction.

We also examined the effects elicited by the combined administration of BP and scopolamine [69]. The retention trial results indicated that 20 mg/kg BP prevents memory acquisition impairment, and that 10 or 20 mg/kg BP prevents memory consolidation impairment, after 1 mg/kg scopolamine administration. These data suggest that the BP antagonistic effects on the scopolamine-induced memory deficits may be caused by the action through both nicotinic and muscarinic receptors [69]. Moreover, the influence of BP on the scopolamine-induced effects can be a result of anticholinergic action and also caused by modulation of the serotoninergic or noradrenergic transmission [3, 9, 72]. In conclusion, BP is able to diminish both the nicotine-induced antiamnestic effects as well as the scopolamine-induced amnestic effects.

Pharmacologic interactions between BP and other psychoactive drugs

Recent studies have shown that BP can facilitate the acquisition of nicotine conditioned place preference (CPP) in rats, suggesting that this antidepressant enhances the rewarding properties of nicotine [14]. In this regard, our subsequent studies were designed to evaluate the involvement of BP in the reinstatement and cross-reinstatement of nicotine-induced CPP in rats. Respect to relapse, the role of BP on the reinstatement of nicotine-induced CPP was investigated by priming dose of other psychoactive drugs (not only nicotine, but also morphine,

ethanol, and cannabinoids) in the CPP test [73, 74]. In these studies, animals are initially trained to associate one distinctive environment with a drug administration and a different environment with a vehicle administration. On the test day, animals mostly spend more time in the drug-paired environment. This acquired preference can be extinguished by pairing administrations of vehicle with both compartments or by letting animals to explore these compartments in the drug absence. After the extinction, a priming dose of the drug of abuse or exposure to a non-drug stimulus reinstates the extinguished CPP. Several animal studies have also reported that drugs other than those previously received can reinstate drug-seeking behavior [68, 75]. This phenomenon, termed cross-reinstatement, has been observed using different classes of drugs.

The testing apparatus for the CPP paradigm is composed of six rectangular chambers, each one divided into two large compartments separated by removable guillotine doors from a small central area. One large compartment is painted white, and the other large compartment is painted black. The central grey area constitutes a "neutral" box, which serves as a connection and a starting chamber. The testing compartments are kept in a sound-proof room with neutral masking noise and dim illumination. The animals' responses are observed on a monitor through a digital video camera system, and the amount of time that the rats spent in each of the two large boxes is recorded using video tracking software. The CPP-reinstatement test takes place on 9 consecutive days and consists of the following phases: preconditioning (pretest), conditioning, post-conditioning (test), extinction and reinstatement.

Our findings revealed that BP pretreatment (10 and 20 mg/kg) inhibits the priming effect of nicotine (0.175 mg/kg, free base) in nicotine-conditioned rats. Interestingly, BP also diminished the priming effects of morphine on nicotine-induced CPP. A statistically effects are observed for both 5 and 10 mg/kg doses of BP. Based on the evidence that BP pre-treatment, especially at the dose of 20 mg/kg, completely abolishes the priming effect of WIN 55,212-2, an ago-antagonist of CB1 cannabinoid receptors, in nicotine-conditioned rats, we concluded that BP diminishes the cannabinoid-induced reinstatement of nicotine-conditioned response. We also showed that BP (5 and 10 mg/kg) blocks the reinstatement of nicotine CPP provoked by ethanol [68].

It has been already reported that BP increases locomotion of freely moving rats, probably through mechanisms involving DA reuptake inhibition and AChRs blockade [18]. In addition, BP potentiates the behavioral locomotor effects of nicotine, and vice versa, nicotine pretreatment facilitates the locomotion mediated by BP [76]. In this regard, Biała's laboratory measured the locomotor activity of rodents in the testing apparatus for the CPP test [73, 74]. The results indicated that BP pretreatment at the highest dose (20 mg/kg) increases locomotor activity in nicotine-conditioned rats primed with nicotine (0.175 mg/kg). Moreover, BP (5 or 10 mg/kg) enhanced the locomotor activity of nicotine-conditioned rats primed with morphine (10 mg/kg). Additionally, BP pretreatment decreased the locomotion of nicotine-conditioned rats primed with WIN 55,212-2 (0.5 mg/kg). A statistically significant decrease in locomotor activity was seen for low doses of BP (10 mg/kg). BP pretreatment (5 and 10 mg/kg) in nicotine-conditioned rats primed with ethanol (0.5 g/kg) also decreased the locomotion. Thus, it can be stated that the efficacy of BP on reinstatement of drug-seeking behaviors might depend on its nonspecific effects on locomotor activity.

The results presented above revealed that BP attenuated or completely blocked the reinstatement of nicotine-induced CPP in rats elicited by a priming dose of either nicotine, morphine, cannabinoids, or ethanol. Therefore, BP can offer a promising approach to the relapse-prevention therapy of addiction, such as nicotine and polydrug abuse. The results obtained in these studies may help to a better understanding of the mechanisms underlying nicotine addiction and the reciprocal relationships between nicotine, cannabis, and ethanol, since co-abuse of these psychoactive compounds is a quite frequent phenomenon. Our findings may further indicate that the cholinergic system plays a pivotal role in the neurobiological processes underlying the relapse to drug addiction. Since reinstatement of drug-seeking plays a key role in the development of dependence, BP may be useful in the relapse-prevention phase of addiction treatment as indicated in the FDA description [54]. In this regard, AChRs can be envisioned as targets for the pharmacological action of new and safer antidepressants as well as for novel anti-addictive compounds.

In summary, whether the actions of BP are mediated by direct actions on dopaminergic and adrenergic transporters and/or AChRs, the central actions of this drug remain to be determined with priority as it represents a promising strategy for different depression- or addiction-related disorders.

Pharmacological activity of BP and its metabolites and derivatives at AChRs

BP acts as a noncompetitive antagonist (NCA) of several AChR subtypes. The pharmacological activities [i.e., inhibitory potency (Table 1) and binding affinities (Table 2)] of BP, its natural metabolites, as well as (±)-SADU-3-72, a photoreactive BP derivative (Figure 1) for different AChR subtypes have been studied by *in vitro* approaches. In addition, functional studies have shown that BP and its analogs inhibit both muscle- and neuronal-type AChRs in the low to intermediate micromolar range [2, 20]. The BP selectivity for different AChRs follows the sequence: α3- >

α4- ~ α1- > α7-containing AChRs (Table 1).

There is emerging evidence that inhibition of different neuronal AChRs by BP may contribute to its dual therapeutic activity either as antidepressant or as anti-nicotinic drug. Ca^{2+} influx results indicate that BP inhibits neuronal hα3β4 AChRs with potency 8-fold higher than that for neuronal hα4β2 AChRs (Table 1). This correlates very well with the evidence that β4-containg AChRs are involved in the antidepressant activity of BP (Figure 2) [27]. Considering the serum levels of BP attained after its oral administration (~0.5–1 μM) [77], the blockade of α3-containing AChRs (i.e., α3β4) seems to be essential. Moreover, it was reported that hydroxybupropion (see molecular structure in Figure 1) has practically the same activity as BP for α3β4* AChRs [78] (Table 1) and can attain ~10-fold higher plasma concentration than that for BP [77], contributing to the clinical efficacy of this drug. Although the inhibitory activity of BP for α7 AChRs is lower compared to that for α4β2 and α3β4 AChRs (~10- to ~50-fold respectively) (Table 1), hippocampal slice experiments indicate that BP may inhibit endogenous α7 AChRs (submitted manuscript).

In order to determine the pharmacologic properties of (±)-SADU-3-72 compared to that for (±)-BP at particular muscle-type AChRs conformational states, binding and functional approaches were used [79-81]. The Ca^{2+} influx results show that (±)-SADU-3-72 is 17- and 6-fold more potent than (±)-BP in inhibiting human (h)α1β1γδ and hα1β1εδ AChRs, respectively (Table 1). To determine the binding affinity of BP and (±)-SADU-3-72 relative to other NCA loci, the influence of these compounds on the maximal binding of either [^{3}H]TCP (the structural and functional analog of phencyclidine (PCP), a known NCA of *Torpedo* AChRs [82] or [^{3}H]imipramine (a tricyclic antidepressant and NCA of AChRs [83]) to *Torpedo* AChRs in different conformational states was determined [79, 80]. Based on [^{3}H]TCP [79] and [^{125}I]SADU-3-72 [81] competition binding experiments, (±)-BP binds with 2-3 times higher affinity to the desensitized AChR compared to the resting AChR (Table 2). These results are in agreement with the [^{3}H]imipramine binding experiments showing that BP discriminates between the desensitized and resting hα3β4 AChRs (manuscript in preparation). The competition binding results also indicated that (±)-SADU-3-72 has high affinity, even higher than (±)-BP, for desensitized and resting states [80, 81]. These results propose that although (±)-BP discriminates between the desensitized and resting states better than (±)-SADU-3-72, the latter is a superior probe for the resting state. The competition binding data also suggest that BP and SADU-3-72 bind at a common site within the resting receptor.

There is evidence showing that Zn^{2+} has antidepressant-like activity [84, 85]. Moreover, Zn^{2+} increases the inhibitory activity of antidepressants on AChRs [86], which seems to be related to the observed enhanced efficacy of several antidepressants in the presence of this cation in animal models of depression [85, 87, 88]. Interestingly, Zn^{2+} potentiates different non-α7 AChRs [85, 89-94]. The results on α4β4 AChRs indicate that the pre-application of BP greatly decreases the potentiating action elicited by Zn^{2+} and that the presence of this cation increases the inhibitory strength of BP [88]. These results corroborate that BP inhibits AChRs in a noncompetitive manner, and open the possibility of allosteric interactions between the positive allosteric modulator site for Zn^{2+} and the NCA site for BP. Based on reports from our and other groups, BP and Zn^{2+} bind to different sites on AChRs. For instance, the allosteric potentiation produced by Zn^{2+} on the $(\alpha4)_3(\beta2)_2$ stoichiometry [i.e., low sensitivity (LS) to agonist activation] is produced by binding to the α4(+)/α4(-) interface at the extracellular domain of the receptor [89, 94]. In particular, α4-His195 on the (-) side of the ACh-binding site and α4-Glu224 on the (+) side of the non-ACh-binding site critically contribute to this potentiation. On the other hand, Zn^{2+} inhibition is mediated by a site located on the β2(+)/α4(-) subunit interface from both $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$ stoichio-metries [i.e., LS and high sensitivity (HS) to agonist activation, respectively]. Additional site-directed mutagenesis and molecular modeling studies on rat α4β2 and α4β4 AChRs suggest that the potentiating effect of Zn^{2+} is mediated by binding to the α4(-)/β2(+) subunit interface (more specifically by coordination with α4-His162 and α4-Glu59) [92, 93, 94]. These results contrast with our findings indicating that the BP binding site is mainly located in the middle of the AChR ion channel (see next section and Figure 3). Unpublished results from our laboratory also suggest that BP can bind to non-luminal sites in the hα4β2 AChRs.

Characterization of the binding sites for BP and SADU-3-72 at AChRs

In silico methods, including comparative/homology modeling and molecular docking simulations, have been employed to study the interactions between BP and SADU-3-72 isomers, in the protonated and neutral states, with distinct AChR subtypes [79, 80, 95]. The docking results indicated that (*R*)-BP and (*S*)-SADU-3-72 interact with the *Torpedo* (Tα1β1γδ), embryonic (hα1β1γδ), and adult (hα1β1εδ) muscle AChRs *via* two different binding modes (Figures 3A and B). In particular, (*R*)-BP and (*S*)-SADU-3-72 bind to overlapping sites located in the ion channel, in a cavity formed between the serine (position 6') and valine (position 13') rings [79, 80]. However, a second binding site located between the outer (position 20') and valine rings was suggested for (*S*)-SADU-3-72 [80] (Figures 3A and B). The results were similar for each enantiomer and for both the protonated and neutral states. The molecular docking results also suggest that the BP enantiomers interact with a

luminal domain overlapping the imipramine locus within the hα4β2 and hα3β4 AChR ion channels, as well as with non-luminal sites located at the transmembrane domain of the hα4β2 AChR (manuscript in preparation).

The computational studies support the concept that certain particular structural features of SADU-3-72 are responsible for the more potent activity compared to (±)-BP (Table 1). The photoreactive azide group (possessing negative and positive charges) at the aromatic ring of each SADU-3-72 enantiomer (see structure in Figure 1) facilitates hydrogen bond formation and polar interactions compared to the BP enantiomers [80]. In particular, the azido group of (*S*)-SADU-3-72 (see Figure 1) presents higher probability of forming a hydrogen bond with α1-Ser248 (serine ring) compared to BP (Figure 3B). In addition, the large halogen (iodine) group of SADU-3-72 at position 3' (see Figure 1) makes this compound more hydrophobic than BP, increasing its polarizability and consequently increasing the possibility of van der Waals interactions with the nonpolar (position 17'), valine, and leucine (position 9') rings. The calculations indicating that the lipophilicity (i.e., logP value) and polarizability values for SADU-3-72 are higher than that for BP support the above hypothesis [80].

Based on the photoaffinity labeling results of [^{125}I]SADU-3-72, two non-overlapping binding sites for (±)-SADU-3-72 and (±)-BP in the *Torpedo* AChR transmembrane domain were identified [81]. One binding site was found within the ion channel, whereas a second site was found near the extracellular end of α1-M1, both depending on the receptor conformational state. In particular, in the resting state, [^{125}I]SADU-3-72 labeled the middle portion of the *Torpedo* AChR ion channel, more specifically at the leucine ring (Figure 3C), which concurs with our docking results (Figures 3A and B). Interestingly, [^{125}I]SADU-3-72 also labeled the desensitiz-ed AChR only at α1-Tyr-213, which is located in a non-luminal site above the extracellular ion channel's mouth (Figure 3C). Interestingly, no other studied NCA interacts with this particular site.

The understanding of the BP activity, alone or in combination with other drugs (e.g., nicotine), at the molecular and behavioral levels, may improve the knowledge of the underlying mechanisms of action. This knowledge is essential for the development of novel BP (or other antidepressant) derivatives with improved clinical profiles for the treatment of depression and psychostimulant-related addictions.

Conflicting interests

The authors have declared that no competing interests exist.

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