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Bupropion and its photoreactive analog (±)-SADU-3-72 interact with luminal and non-luminal sites at human α4β2 nicotinic acetylcholine receptors

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ABSTRACT

The interaction of (±)-bupropion [(±)-BP] with the human (h) α4β2 nicotinic acetylcholine receptor (AChR) was compared to that for its photoreactive analog (±)-2-(*N*-*tert*-butylamino)-3′-iodo-4′-azidopropiophenone [(±)-SADU-3-72]. Ca^{2+} influx results indicated that (±)-SADU-3-72 and (±)-BP inhibit hα4β2 AChRs with practically the same potency. However, (±)-SADU-3-72 binds to the [^{3}H]imipramine sites at resting and desensitized hα4β2 AChRs with 3-fold higher affinity compared to that for (±)-BP, which is supported by molecular docking results. The docking results also indicate that each isomer of BP and SADU-3-72, in the protonated state, interacts with luminal and non-luminal sites. In the channel lumen, both ligands bind to two overlapping subsites, one that overlaps the imipramine site, and another much closer to the cytoplasmic side. The results suggest, for the first time, three differentiated non-luminal domains, including the transmembrane (TMD), extracellular (ECD), and ECD-TMD junction. In the ECD-TMD junction, BP and SADU-3-72 bind to overlapping sites. Interestingly, only SADU-3-72 binds to intrasubunit and intersubunit sites in the TMD, and to additional sites in the ECD. Our results are consistent with a model where BP and SADU-3-72 bind to overlapping sites in the luminal and ECD-TMD junctional domains of the hα4β2, whereas only SADU-3-72 binds to additional non-luminal sites. The BP junctional site opens the door for additional inhibitory mechanisms. The pharmacological properties of (±)-SADU-3-72 showed in this work support further photolabeling studies to mapping the BP binding sites in the hα4β2 AChR.

1. Introduction

(±)-Bupropion [(±)-BP; (±)-2-(*tert*-butylamino)-1-(3-chlorophenyl)propan-1-one] (see the molecular structures of the (*S*)-(+)- and (*R*)-(−)-enantiomers in Fig. 1) was originally developed as an antidepressant (Wellbutrin®) but is also currently used as a smoking cessation aid (Zyban®) (reviewed in (Arias, 2009; Arias et al., 2014)). It is believed that the antidepressant activity of (±)-BP is due to its inhibitory action on the dopamine and norepinephrine reuptake systems. However, new evidence suggests that the antidepressant and anti-addictive properties of (±)-BP are also due to the noncompetitive antagonistic activity on several nicotinic acetylcholine receptors (AChRs) (reviewed in (Arias, 2009; Arias et al., 2014)). AChRs are members of the Cys-loop ligand-gated ion channel superfamily that include type A and C γ-aminobutyric acid, type 3 5-hydroxytryptamine (serotonin) (i.e., 5-HT_3R), and glycine receptors (reviewed in (Arias, 2006; Kamens et al., 2011)).

The structural characterization of the BP binding sites in neuronal AChRs is relevant for the development of novel BP

Abbreviations: AChR, nicotinic acetylcholine receptor; NCA, noncompetitive antagonist; (±)-BP, (±)-bupropion [(±)-2-(*tert*-butylamino)-1-(3-chlorophenyl) propan-1-one]; (±)-SADU-3-72, (±)-2-(*N*-*tert*-butylamino)-3′-iodo-4′-azidopropiophenone; ECD, extracellular domain; TMD, transmembrane domain; H-bond, hydrogen bond; MD, molecular dynamics; RMSD, root mean square deviation; AVG, average RMSD value; VAR, RMSD variance; κ-BTx, κ-bungarotoxin; RT, room temperature; BS, binding saline; K_i, inhibition constant; K_d, dissociation constant; IC_{50}, ligand concentration that inhibits 50% binding or Ca^{2+} influx; n_H, Hill coefficient; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum.

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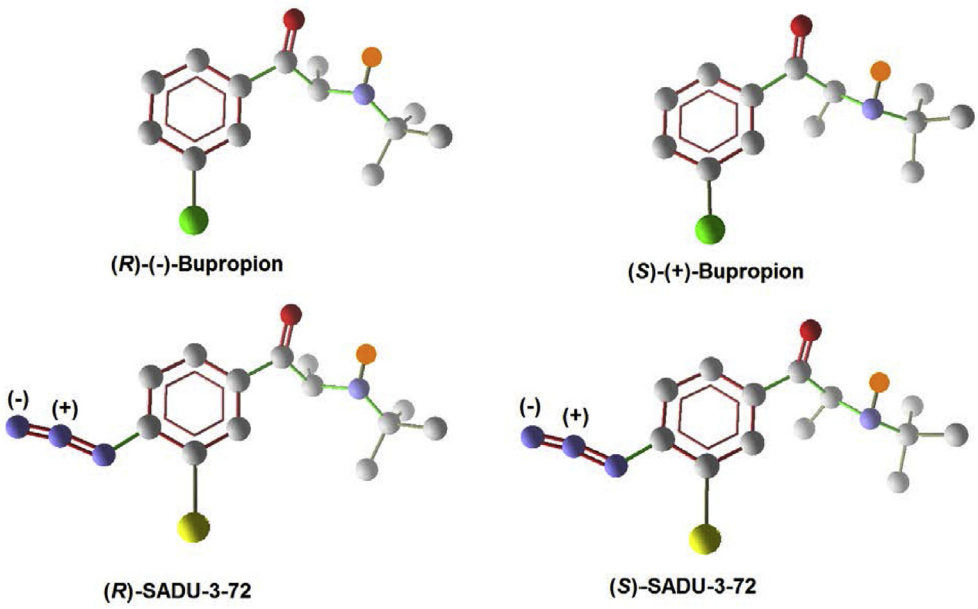

Fig. 1. Molecular structure of (*S*)-(+)- and (*R*)-(−)-bupropion [(*S*)-(+)- and (*R*)-(−)-2-(*tert*-butylamino)-1-(3-chlorophenyl)propan-1-one], and its photoreactive analogs (*S*)- and (*R*)-SADU-3-72 [(*S*)- and (*R*)-2-(*N-tert*-butylamino)-3'-iodo-4'-azidopropiophenone]. The chloride atom is rendered in green, iodine in yellow, carbon in gray, nitrogen in blue, oxygen in red, and hydrogen in orange. In SADU-3-72, the iodine atom (yellow) and the photoreactive azide group (blue), possessing negative and positive charges, are located at positions 3′ and 4′, respectively, on the aromatic ring. The ligands are shown in the ball and stick mode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

derivatives with improved clinical profiles for treating depression, nicotine addiction, and psychostimulant abuse. In this regard, a photoreactive analog of (±)-BP, (±)-2-(*N-tert*-butylamino)-3'-iodo-4'-azidopropiophenone [(±)-SADU-3-72] (see molecular structures of the (*S*)- and (*R*)-enantiomers in Fig. 1), has been developed for photoaffinity labeling studies (Lapinsky et al., 2012). The pharmacological and structural features of (±)-SADU-3-72 (Arias et al., 2012) and the photolabeling of the BP binding sites in the resting and desensitized states were recently studied in muscle AChRs (Pandhare et al., 2012). Following the characterization of BP interacting with neuronal AChRs, which is more clinically relevant, we determined, in this work, the pharmacological properties of (±)-SADU-3-72 at the α4β2 AChR, the most abundant AChR in the brain, with the purpose of laying the basis for further photoaffinity labeling studies. In this regard, the functional and structural properties of BP interacting with this AChR subtype are compared to that for SADU-3-72 by means of Ca^{2+} influx, [^{3}H]imipramine binding, and molecular modeling studies. For the molecular modeling studies, we took advantage of the new crystallographic structure of the homopentameric mouse (m) 5-HT_{3A}R at 3.5 Å resolution (PDB ID: 4PIR) (Hassaine et al., 2014) to build the human (h) α4β2 AChR by homology modeling. Considering that the interaction of imipramine with hα4β2 AChRs has been previously characterized (Arias et al., 2010a), [^{3}H]imipramine competition binding assays are performed to determine the interaction of (±)-BP and (±)-SADU-3-72 with these binding sites. The results from the present work give information about the interaction of BP with hα4β2 AChRs in different conformational states, and will pave the way for further photolabeling studies using (±)-SADU-3-72 as a probe for the BP sites at the α4β2 AChR.

2. Methods and materials

2.1. Ca^{2+} influx measurements in the HEK293-hα4β2 cell line

The HEK293-hα4β2 cell line was cultured as described previously (Arias et al., 2010a, 2013a,b, 2015). Under these conditions, the AChRs are predominantly expressed with the $(\alpha4)_3(\beta2)_2$ stoichiometry (Arias et al., 2013b). Ca^{2+} influx experiments were performed as previously described (Arias et al., 2010a, 2013a,b, 2015). Briefly, 5×10^4 cells per well were seeded 48 h prior to the Ca^{2+} influx experiment on black poly-L-lysine 96-well plates (Costar, Corning Inc., New York, USA) and incubated at 37 °C in a humidified atmosphere (5% CO_2/95% air). 16 h before the experiment, the medium was changed to 1% bovine serum albumin (BSA) in HEPES-buffered salt solution (HBSS) (130 mM NaCl, 5.4 mM KCl, 2 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.9 mM NaH_2PO_4, 25 mM glucose, 20 mM HEPES, pH 7.4). On the day of the experiment, the medium was removed by flicking the plates and replaced with 100 μL HBSS/1% BSA containing 2 μM Fluo-4 (Molecular Probes, Eugene, OR, USA) in the presence of 2.5 mM probenecid (Sigma, Buchs, Switzerland). The cells were then incubated at 37 °C in a humidified atmosphere (5% CO_2/95% air) for 1 h. Plates were flicked to remove excess of Fluo-4, washed twice with HBSS/1% BSA, and finally refilled with 100 μL of HBSS containing different concentrations of BP or (±)-SADU-3-72, and pre-incubated for 5 min. Plates were then placed in the cell plate stage of the fluorimetric imaging plate reader (Molecular Devices, Sunnyvale, CA, USA). A baseline consisting of 5 measurements of 0.4 s each was recorded. (±)-Epibatidine (0.1 μM) was then added from the agonist plate to the cell plate using the 96-tip pipettor simultaneously to fluorescence recordings for a total length of 3 min. The laser excitation and emission wavelengths were 488 and 510 nm, at 1 W, with a CCD camera opening of 0.4 s.

2.2. [^{3}H]Imipramine binding experiments using hα4β2 AChRs in different conformational states

The effect of (±)-BP and (±)-SADU-3-72 on [^{3}H]imipramine binding to hα4β2 AChRs in different conformational states was studied as described previously (Arias et al., 2010a, 2013a,b). In this regard, hα4β2 nAChR membranes (1.5 mg/mL) were suspended in