Biochemical Pharmacology 78 (2009) 1035–1042

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Dimebolin is a 5-HT_6 antagonist with acute cognition enhancing activities

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ARTICLE INFO

Article history:
Received 11 May 2009
Accepted 15 June 2009

Keywords:
Dimebolin
Dimebon
5-HT_6
Cognition
Alzheimer's disease

ABSTRACT

Dimebolin (Dimebon™), is a non-selective antihistamine approved in Russia for the treatment of allergy. Recently, this drug has been shown to be neuroprotective in cellular models of Alzheimer's disease and Huntington's disease, and to preserve cognitive function when chronically administered to AF64A lesioned rats. Interests in identifying the molecular targets of dimebolin have intensified with reports of efficacy in clinical trials with Alzheimer's patients. Dimebolin has been found to interact with a number of molecular targets including acetylcholinesterases, N-methyl-D-aspartate receptors, and voltage-gated calcium channels, with potencies in the range of 5–50 μM. In the present study, the action of dimebolin at the serotonin 5-HT_6 receptor was investigated. Dimebolin binds with moderate affinity to both the human and rat recombinant 5-HT_6 receptor (K_i = 26.0 ± 2.5 nM and 119.0 ± 14.0 nM respectively) as well as the native rat 5-HT_6 receptor, and acts as an antagonist in functional cAMP assays. Furthermore, dimebolin occupies the 5-HT_6 receptor *in vivo* as assessed by *ex vivo* autoradiography, with a dose–occupancy relationship similar to that of the selective 5-HT_6 antagonist SB-399885. Finally, both SB-399885 and dimebolin produce an acute enhancement of short-term social recognition memory, although dimebolin is approximately 10-fold less potent than SB-399885. Taken together, these studies demonstrate that dimebolin antagonizes the 5-HT_6 receptor with higher affinity than other targets characterized to date, and suggest that this activity may play a role in the acute cognition enhancing effects of this compound in preclinical models and in the clinic.

1. Introduction

Dimebolin hydrochloride (Dimebon™; (2,8-Dimethyl-5-[2-(6-methylpyridin-3-yl)ethyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole dihydrochloride) was originally approved in Russia as a non-selective antihistamine for the treatment of skin allergy and allergic rhinitis [1] and was withdrawn from the market with the advent of more selective treatments. Interestingly, dimebolin has been found to be neuroprotective against the β-amyloid fragment Aβ 25–35 in cultures of cerebellar granule cells, an *in vitro* model that may mimick some of the neurodegenerative aspects of Alzheimer's disease (AD) [2]. Dimebolin has also been demonstrated to produce neuroprotection from glutamate-induced apoptosis in striatal neuronal cultures derived from a Huntington's disease mouse model [3]. In addition, dimebolin preserves learning and memory in an active avoidance conditioned learning task after chronic administration in a preclinical AF64A cholinergic-lesion model [2,4], however it is not known if the cognitive benefit reported in these studies is due to a neuroprotective effect or acute enhancement of cognition.

Recently, dimebolin has attracted renewed interest after demonstration of significant efficacy in a double blind placebo-controlled 26 week trial in which the compound was found to have cognition enhancing effects in persons suffering from mild to moderate Alzheimer disease (AD) as assessed by the ADAS-cog battery [5]. To date the precise mechanism of action whereby dimebolin exerts its procognitive and neuroprotective effects is unknown. Dimebolin exhibits a rich pharmacological profile and is known to be a weak (IC_{50} = 8–42 μM) acetylcholinesterase inhibitor [2], an N-methyl-D-aspartate (NMDA) receptor antagonist (IC_{50} = 10 μM) [3,6], an inhibitor of voltage-gated calcium channels (IC_{50} = 50 μM) [7], and a modulator of the mitochondrial permeability transition pore (10–200 μM) [8]. Furthermore, dimebolin has been shown to interact with a number of additional targets in a standard lead profiling panel, inhibiting 18 receptors by more than

Abbreviations: Dimebolin, 2,8-Dimethyl-5-[2-(6-methylpyridin-3-yl)ethyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole dihydrochloride; SB-399885, (N-(3,5-Dichloro-2-methoxyphenyl)-4-methoxy-3-piperazin-1-yl-benzenesulfonamide hydrochloride; SB-258528, 4-Iodo-N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]benzenesulfonamide; NMDA, N-methyl-D-aspartate receptors; 5-HT, serotonin; AD, Alzheimer disease; ANOVA, analysis of variance.

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doi:10.1016/j.bcp.2009.06.021

50% at a 10 μM concentration, including a number of serotonin (5-HT) receptors [3]. These findings suggest that dimebolin will prove to be a more potent ligand at a number of additional targets.

5-HT has been implicated in a number of psychiatric disorders including depression, anxiety, obesity, and sleep disturbances [9,10]. Recently, attention has focused on the role of 5-HT in learning and memory [11]. While several 5-HT receptors are implicated in learning and memory processes [12], the 5-HT_6 receptor is somewhat unusual in that it appears to influence long-term memory with little role in other behaviors such as anxiety [13]. 5-HT_6 receptors are predominately expressed in the central nervous system notably in the cerebral cortex, striatum, hippocampus, and nucleus accumbens [14–16]. With the development of potent and selective 5-HT_6 receptor antagonists, efficacy in a number of preclinical cognitive models has been reported [17–19]. More recently it has been reported that the selective 5-HT_6 receptor antagonist, SB-742457 demonstrated procognitive efficacy in a placebo-controlled double blind phase II trial in mild to moderate AD patients [18].

Given dimebolin's cognition enhancing effects, and the finding that it interacts with a number of 5-HT receptors, we hypothesized that dimebolin could bind to the 5-HT_6 receptor. Here we report that dimebolin is a moderately potent 5-HT_6 antagonist *in vitro* in both recombinant and native assays, and *in vivo* as assessed by an *ex vivo* receptor occupancy assay. Consistent with its activity at the 5-HT_6 receptor [20], dimebolin improves short-term working memory in a rat social recognition model. We conclude that inhibition of the 5-HT_6 receptor is an additional pharmacological activity of dimebolin which may play an important role in the apparent clinical efficacy of this compound.

2. Materials and methods

2.1. Chemicals

5-HT, mianserin and methiothepin were obtained from Sigma–Aldrich (St. Louis, MO). Dimebolin hydrochloride (Dimebon™; (2,8-Dimethyl-5-[2-(6-methylpyridin-3-yl)ethyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole dihydrochloride) was purchased from Biotrend (Cologne, Germany). SB-399885 (N-(3,5-Dichloro-2-methoxyphenyl)-4-methoxy-3-piperazin-1-yl-benzenesulfonamide hydrochloride) was obtained from Tocris (Ellisville, MO). Unless otherwise noted, all other chemicals and reagents were obtained from Sigma–Aldrich (St. Louis, MO).

2.2. Animals

Juvenile (80–130 g) and adult male Sprague Dawley rats (350–450 g) were obtained from Charles River (Wilmington, MA). All animals were maintained on a 24 h light/dark cycle (on at 7 am/off at 7 pm), with food and water available *ad libitum*. All experimental animal procedures were conducted in accordance with the National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of Cephalon, Inc.

2.3. Membrane preparation

Membranes were prepared from CHO-K1 cells stably transfected with the human 5-HT_6 receptor or HEK293 cells stably expressing a rat 5-HT_6 receptor. The cells were grown in Gibco Advanced DMEM-F12 containing 2% dialyzed FBS (Hyclone, Logan, UT). The cells were harvested in phosphate buffered saline (PBS) containing 0.1 mM EDTA and pelleted by centrifugation (1000 × *g*), the supernatant was discarded and the pellets were stored at −80 °C prior to membrane preparation. Membranes were prepared as previously described [21]. Briefly, frozen cell pellet was resuspended in a lysis buffer containing 5 mM Tris–HCl (pH 7.5), 5 mM EDTA and 1 complete EDTA-free protease inhibitor tablet (Roche Applied Science, Indianapolis, IN) per 50 ml buffer, and homogenized with a tissue homogenizer. The cell lysate was then centrifuged at 40,000 × *g* for 30 min at 4 °C to collect the membranes. The membrane pellets were washed in membrane buffer containing 50 mM Tris–HCl (pH 7.5), 0.6 mM EDTA, 5 mM $MgCl_2$, 1 complete EDTA-free protease inhibitor tablet per 50 ml buffer using a tissue homogenizer. The membranes were centrifuged at 40,000 × *g* for 30 min at 4 °C and the pellets were resuspended in membrane buffer containing 250 mM sucrose, and protein concentration was determined using the Coomassie Plus kit (Pierce Biotechnology, Rockford, IL).

2.4. Receptor binding assays

Membranes prepared from cells expressing recombinant human 5-HT_6 (h5-HT_6) and rat 5-HT_6 (r5-HT_6) receptors were resuspended in assay buffer containing 50 mM Tris–HCl, (pH 7.4), 4 mM $CaCl_2$, 10 μg/ml saponin, and 0.1% (w/v) ascorbic acid and incubated for 3 h at room temperature with 4 nM [^{3}H]LSD (PerkinElmer, Boston, MA, cat # NET638), vehicle or test compound, and either 0.25 mg/well for h5-HT_6 or 0.5 mg/well for r5-HT_6 FlashBlue scintillation beads (PerkinElmer, Boston, MA, cat. # FB001) in a final volume of 80 μl in a 96-well plate. Membranes were used at 1.75 μg per well and 4 μg per well for h5-HT_6 and r5-HT_6 binding, respectively. Membranes and beads were pre-incubated at 4 °C for 30 min prior to addition of test compounds. Test compounds or assay controls for total and non-specific binding were diluted in DMSO as 100× solutions and serially diluted by half log concentrations on a PerkinElmer JANUS Automated Workstation. Serotonin (10 μM for h5-HT_6) or mianserin (100 μM for r5-HT_6) was used to determine non-specific binding in the assay. Plates were read using the Microbeta Trilux 1450 LSC and luminescence counter.

Binding of [^{3}H]LSD to the h5-HT_6 membranes was saturable with B_{max} = 19.6 ± 4.7 pmol/mg protein and K_d = 4.5 ± 1.1 nM. The r5-HT_6 cell line expressed approximately 3.3 ± 1.8 pmol/mg protein binding sites for [^{3}H]LSD with K_d = 4.8 ± 0.7 nM.

2.5. cAMP measurement

cAMP level in the cells were determined as previously described [22] with minor modifications using a homogenous time-resolved fluorescent (HTRF®) assay (Cisbio, Bedford, MA) in 1321N1 human astrocytoma cell line stably expressing the h5-HT_6 receptor or HEK293 transiently expressing the r5-HT_6 receptor. Frozen cells were thawed and washed with assay media (DMEM with 10% FBS). Cells were plated at a density of 5000 cells per well in white 384-well OptiPlates (PerkinElmer Life Sciences, Boston, MA). Test compounds in DMSO were added to cells in a volume of 100 nl, and the plate was pre-incubated at room temperature for 10 min (final DMSO concentration was 0.5%). The cells were then stimulated with 10 nM 5-HT (an EC_{80} concentration) for 30 min at room temperature. The reaction was stopped by the addition of the 12 μl of cAMP-d2 in lysis buffer, followed by addition of 12 μl of anti cAMP-Cryptate in lysis buffer. The plate was incubated at room temperature for 1 h and read on multi-labeled plate reader EnVision 2102 or 2104 (PerkinElmer Life Sciences, Boston, MA). The fluorescence ratio (665 nm/590 nm × 10^4) was calculated, which was inversely proportional to the level of cAMP in the sample.

2.6. Data analysis from in vitro studies

The inhibition curves were fitted with a four parameter logistic equation giving IC_{50} values using Prism 4 (GraphPad Software Inc.,

San Diego, CA). K_i values from competition binding assays and $K_{b,app}$ values from the cAMP assay were determined by the method of Cheng and Prusoff [23].

2.7. Brain slices

To generate slices used in all autoradiography studies, animals were culled by rapid decapitation and the brains were quickly removed and frozen in dry-ice cooled isopentane and stored at −80 °C until use. For *ex vivo* occupancy studies, animals were first injected with vehicle or test compound as detailed below, and culled 1 h following the injection. Coronal sections through the rat brain were cut at a thickness of 20 μm on a Leica CM3050 S cryostat (Leica Micosystem Inc., Bannockburn, IL), thaw mounted onto gelatin coated slides (Superfrost plus, Fisher Scientific, Newark, DE) and stored at −80 °C until used.

2.8. Rat brain slice autoradiography in vitro

Brain slices ($N = 2$ animals/dose, repeated in three independent experiments for total $N = 6$ animals/data point) on microscope slides generated as described above were dried under a stream of air and incubated with 0.25 nM [^{125}I]SB-258585 (PerkinElmer, specific activity 2200 Ci/mmol) and various concentrations of the test compound in binding buffer containing 50 mM Tris–HCl (pH 7.4) 5 mM $MgCl_2$, 0.5 mM EDTA, 10 mM pargyline and 0.1% ascorbic acid. The reaction was stopped by rinsing the slides three times in cold washing buffer containing 50 mM Tris–HCl (pH 7.4) for 30 min each and then dipping in water in order to remove buffer salt. The slides were dried under a stream of air overnight before quantitative analysis. Non-specific binding was determined in the adjacent sections by the addition of a saturating (10 μM) concentration of methiothepin.

2.9. Ex vivo occupancy in rat brain

Animals ($N = 2$ animals/dose, repeated in three independent experiments for total $N = 6$ animals/data point) treated with vehicle (0.5% methylcellulose/0.2% Tween-80), 0.1–100 mg/kg i.p. SB-399885, or 0.1–30 mg/kg i.p. dimebolin were culled as described above 1 h post-treatment. The rats were sacrificed and brain cryostat slices were mounted on microscope slides, dried, and subjected to binding reactions with [^{125}I]SB-258585 as described above. Non-specific binding was defined in animals treated with a 100 mg/kg i.p. dose of SB-399885.

2.10. Quantitative analysis with β-imager

After the completion of the binding reaction and wash steps, sections were dried overnight at room temperature under a stream of air. Radioactivity was quantified using a β-imager (Biospace, France) as previously described [24]. The striatum was chosen as the region of interest for the quantitative assessment of 5-HT_6 receptor binding based on the relatively high expression of 5-HT_6 in this brain region [14], and the fact that the striatum is a large and relatively homogenous structure which allows for reliable and repeatable quantification by autoradiographic methods. All analysis was performed on sections corresponding to coordinates AP 11.16 to 8.64 mm from the interaural line from the atlas of Paxinos and Watson [25]. Data from brain sections were collected over 15 h. The levels of bound radioactivity in the striatum from each coronal section were directly determined by counting the number of β-particles emerging from the delineated area by using the Beta vision program (BioSpace). Consequently, the radioligand binding signal was expressed in counts per minute per square millimeter. Each treated animal was read in duplicate (two coronal sections for each animal). The inhibition of specific [^{125}I]SB-258585 binding was determined in order to provide an indication of receptor occupancy by the compound. Total specific binding (TBS) = total binding − non-specific binding; individual specific binding = individual counts − non-specific. The percentage of inhibition of specific binding was calculated as follows: (individual specific binding/mean total specific binding) × 100. The obtained values were plotted as a curve and represent the mean ± S.E.M. of 3 individual experiments run in duplicate ($n = 6$). The dose–response curve was fit to a four parameter logistic equation to yield an IC_{50} value using Prism 4.0 (GraphPad Software Inc., San Diego, CA).

A least squares linear regression model as implemented in the Prism 4.0 package (GraphPad Software Inc., San Diego, CA) was used to fit data on the relationship between receptor occupancy and plasma or brain concentration of compound and to interpolate the brain concentration of each compound producing a 50% occupancy (OC_{50}). A linear model was used rather than the theoretically more appropriate sigmoidal model due to limitations on quantification at the extremes of the concentration–occupance relationship. At the lower end of the relationship, the detection methods for both receptor occupancy and measurement of compound in brain and plasma samples sets practical limits on how well the curve can be defined. At the upper end, issues of compound solubility and imperfect dose escalation limit the definition of a true maximum. Under these circumstances a linear correlation provides the most conservative estimate of the relationship between compound concentration and receptor occupancy.

2.11. Rat social recognition model

Social recognition was performed as previously described [26,27] with minor modifications. Adult male rats (Sprague Dawley, 350–450 g) were exposed to a male juvenile rat (Sprague Dawley 80–130 g) for a 5 min trial (Trial 1) and, after an inter-exposure interval (IEI) of 120 min, the same juvenile rat was returned to the test box with the adult rat for a second 5 min exposure (Trial 2). Following short IEIs (15–30 min), adult rats investigated the juvenile for significantly less time during Trial 2 relative to Trial 1, indicating that the adult rat retained memory for the juvenile. Memory of the juvenile was lost as the IEI was increased, with a 2 h IEI producing relatively equal exploration on both the first and second trials resulting in a ratio of investigation duration (RID) near 1. Rats were dosed i.p. with vehicle (0.5% methylcellulose (w/v):0.2% Tween-80), SB-399885 or dimebolin immediately after the first trial providing a pre-treatment time of 120 min prior to the second trial. Since this assay relies on natural social behavior which is easily disrupted by a number of environmental factors, it is essential to run control animals on every experimental day in order to be confident that normal behavior is intact. As this is a relatively low throughput assay with each compound run over a number of weeks, a larger number of animals accumulate in the control group leading to a large range of *N*-values. Separate sub-group analysis and test of equal variance were performed to insure that the higher number of control animals did not introduce a bias in the results. Additional controls included a separate group of rats that received effective doses of compound and were subsequently exposed to a novel juvenile on the second exposure.

All values are expressed as the mean ± S.E.M. Data were analyzed by analysis of variance (1-way ANOVA) using dose as a factor and Dunnett's *post hoc* test [GraphPad Prism version 4.03].

3. Results

3.1. Dimebolin binds to the human and rat 5-HT$_6$ receptor

Based on the reported interaction of dimebolin (10 μM) with multiple 5-HT receptors including 5-HT$_6$ [3], and the known cognition enhancing properties of 5-HT$_6$ receptor antagonists, it was hypothesized that dimebolin may be a potent 5-HT$_6$ receptor antagonist. Validation of the radioligand binding assay in membranes containing recombinantly expressed receptors using the selective 5-HT$_6$ antagonist SB-399885 [20] demonstrates displacement of [^{3}H]LSD binding with K_i values in good agreement with published data [20]. SB-399885 displaced [^{3}H]LSD binding at both the human (K_i = 1.2 ± 0.2 nM) and the rat (K_i = 1.5 ± 0.4 nM) 5-HT$_6$ receptor. Interestingly, dimebolin was found to displace [^{3}H]LSD in a concentration-dependent manner with K_i values of 26 ± 2.5 nM at the human 5-HT$_6$ receptor, and 119 ± 14 nM at the rat 5-HT$_6$ receptor (Table 1, Fig. 1). While dimebolin binds to 5-HT$_6$ with lower affinity than SB-399885, it is interesting to note that the compound binds with an equal (rat) or greater (human) affinity when compared to the endogenous ligand 5-HT (Table 1).

In order to confirm interaction with natively expressed 5-HT$_6$ receptors, dimebolin was tested for displacement of [^{125}I]SB-258585 binding in rat brain slices. Autoradiographic analysis of coronal rat brain sections labeled *in vitro* with [^{125}I]SB-258585 reveals that both the selective 5-HT$_6$ antagonist SB-399885 and dimebolin displaced the specific binding of the ligand with IC$_{50}$ values of 7.5 ± 4 and 210.0 ± 42 nM, respectively (Table 1, Fig. 1C).

Having demonstrated binding to the 5-HT$_6$ receptor, dimebolin was next tested in functional assays in order to determine if it acts as an antagonist. 5-HT mediates a concentration-dependent accumulation of cAMP in 1321N1 human astrocytoma cells, with an EC$_{50}$ of 2.3 ± 0.4 nM (data not shown). Consistent with mediation by the human 5-HT$_6$ receptor, the 5-HT-induced cAMP accumulation was dose-dependently inhibited by the selective 5-HT$_6$ antagonist SB-399885 (Fig. 2). Dimebolin also produced a dose-dependent decrease of 5-HT-induced cAMP production exhibiting an $K_{b,app}$ = 23.1 ± 0.5 nM (Fig. 2, Table 1) suggesting that this compound is a functional antagonist of the h5-HT$_6$ receptor. Studies in HEK293 cells transiently expressing the r5-HT$_6$ receptor found similar antagonism of the rat receptor with both SB-399885 ($K_{b,app}$ = 0.96 ± 0.1 nM) and dimebolin ($K_{b,app}$ = 44 ± 2.4 nM) functioning as antagonists at the rat receptor (Fig. 2B, Table 1).

Table 1
Binding affinity (K_i or IC$_{50}$) and functional potency ($K_{b,app}$) of SB-399885 and dimebolin at human and rat recombinant and native rat 5-HT$_6$ receptors.

	Binding			Function	
	h5-HT$_6$ K_i (nM)	r5-HT$_6$ K_i (nM)	Native r5-HT$_6$ IC$_{50}$ (nM)	h5-HT$_6$ $K_{b,app}$ (nM)	r5-HT$_6$ $K_{b,app}$ (nM)
5-HT	215 ± 38.0	127 ± 20.0	–	–	–
SB-399885	1.2 ± 0.2	1.47 ± 0.4	7.5 ± 4.0	1.4 ± 0.2	0.96 ± 0.1
Dimebolin	26 ± 3.0	119 ± 14.0	210 ± 42.0	23 ± 0.5	44 ± 2.4

Data are expressed as mean ± S.E.M. obtained from three independent experiments.

3.2. Dimebolin occupies the 5-HT$_6$ receptor in vivo

Having found dimebolin to be an antagonist of the human and rat 5-HT$_6$ receptor, the compound was next tested for the ability to antagonize receptors *in vivo* as estimated using an *ex vivo* binding assay. As shown in Fig. 3A and B, SB-399885 dosed *in vivo* (0.1–100 mg/kg i.p.) produced a dose-dependent inhibition in *ex vivo* [^{125}I]SB-258585 binding to coronal brain slices obtained from treated rats. Statistical analysis revealed that the SB-399885-induced reduction in total specific binding measured *ex vivo* reached significance at the 3–100 mg/kg i.p. dose (ANOVA

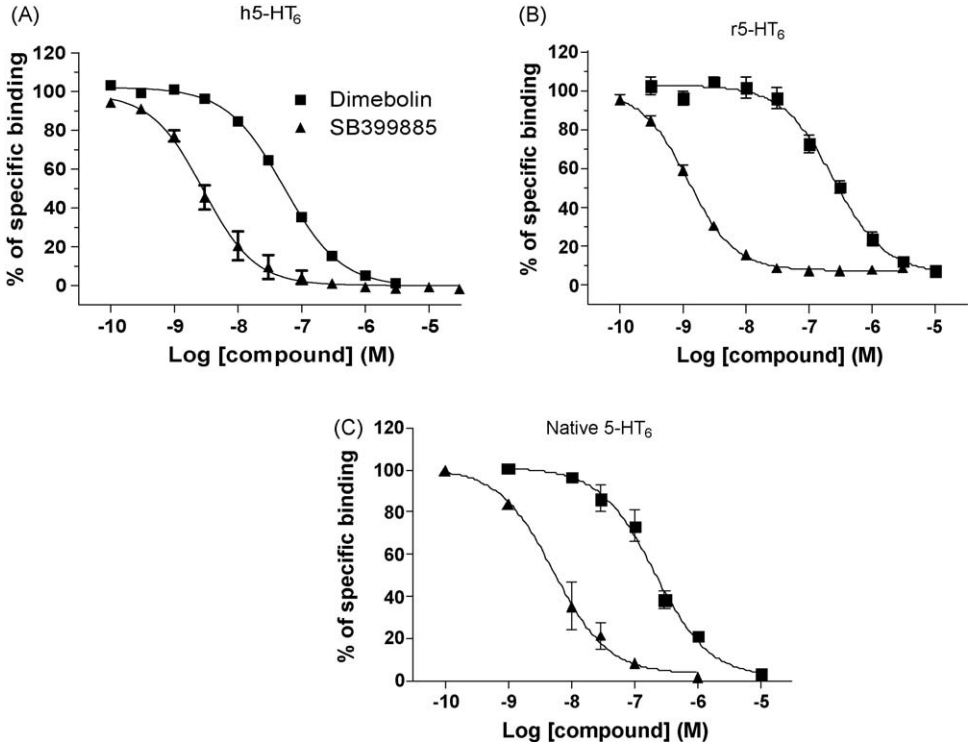

Fig. 1. Pharmacological profile of SB-399885 (▲) and dimebolin (■) in SPA binding format using CHO/h5-HT$_6$ (A) or HEK292/r5-HT$_6$ (B) membranes. Membranes were incubated with [^{3}H]LSD as described in Section 2. Data are the mean ± S.E.M. of 3–6 independent experiments. Average K_i values are given in Table 1. (C) Binding of SB-399885 (▲) and dimebolin (■) in coronal rat brain slices assessed by *in vitro* autoradiography. Slices were incubated with [^{125}I]SB-258585 as described in Section 2. Data points represent the mean ± S.E.M. of three independent experiments.

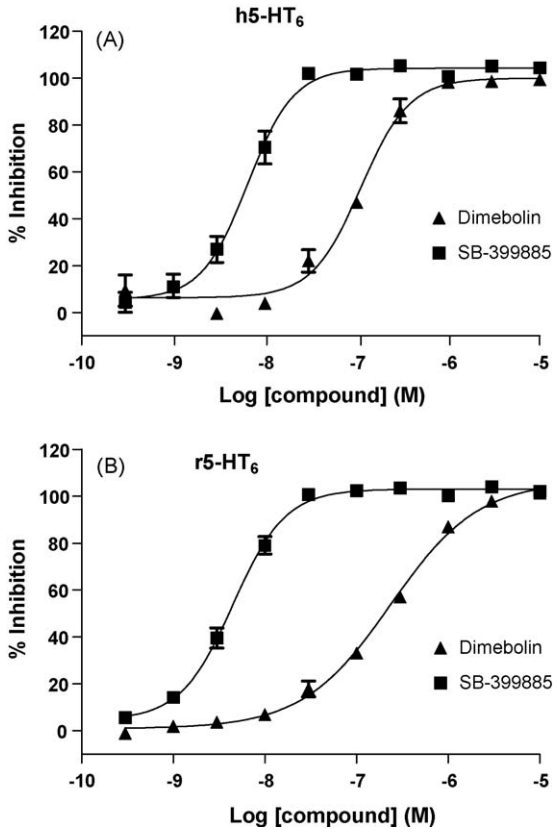

Fig. 2. Effect of SB-399885 (▲) and dimebolin (■) on 5-HT-stimulated cAMP accumulation in a 1321N1 human astrocytoma cell line stably expressing the h5-HT_6 receptor (A) or HEK293 transiently expressing the r5-HT_6 receptor (B). Data points are expressed as percentage of inhibition and represent the mean ± S.E.M. of four independent experiments.

$F_{7,37} = 5.90$; $p < 0.05$ Dunnett's *post hoc*). Dimebolin (0.1–30 mg/kg i.p.) also produced a dose-dependent decrease in *ex vivo* [^{125}I]SB-258585 binding (Fig. 3A and C). Statistical analysis revealed that the dimebolin-induced reduction in total specific binding measured *ex vivo* reached significance at the 10 and 30 mg/kg i.p. dose (ANOVA $F_{6,36} = 34.49$; $p < 0.05$ Dunnett's *post hoc*). The data obtained for each compound were fit with a four parameter logistic equation to yield an ID_{50}. The ID_{50} values (mean ± S.E.M.) were 9 ± 2.5 mg/kg i.p. for SB-399885, and 8 ± 0.5 mg/kg i.p. for dimebolin. The plasma and brain concentrations of both compounds were determined by liquid chromatography/mass spectrometry. A linear correlation exists between the inhibition of [^{125}I]SB-258585 binding and plasma and brain concentration (Fig. 4). These data were used to interpolate the brain concentration of each compound producing a 50% occupancy (OC_{50}, Table 2).

3.3. Dimebolin improves short-term memory

As discussed above, 5-HT_6 antagonists are known to produce pro-cognitive effects in a number of preclinical models. The findings that dimebolin acts as a 5-HT_6 antagonist *in vitro* and occupies the 5-HT_6 receptor *in vivo* led to the hypothesis that dimebolin may produce an acute enhancement of short-term memory in part by blocking the 5-HT_6 receptor. We therefore compared the effect of the selective 5-HT_6 antagonist SB-399885 with that of dimebolin in the rat social recognition model of short-term memory [26,27]. As described in Section 2, adult animals were exposed to a juvenile rat and allowed to investigate the juvenile for 5 min. Using the *ex vivo* occupancy data to guide the selection of a starting dose, adult animals were dosed with either SB-399885 (0.01–3 mg/kg i.p.), or dimebolin (1–30 mg/kg i.p.) immediately after the first trial, and retested for recognition of the juvenile 120 min later.

As presented in Fig. 5A, SB-399885 produced a significant reduction in RID at the 1 and 3 mg/kg i.p. dose (ANOVA $F_{7,168} = 4.37$; $p < 0.05$ Dunnett's *post hoc*). The same doses of SB-

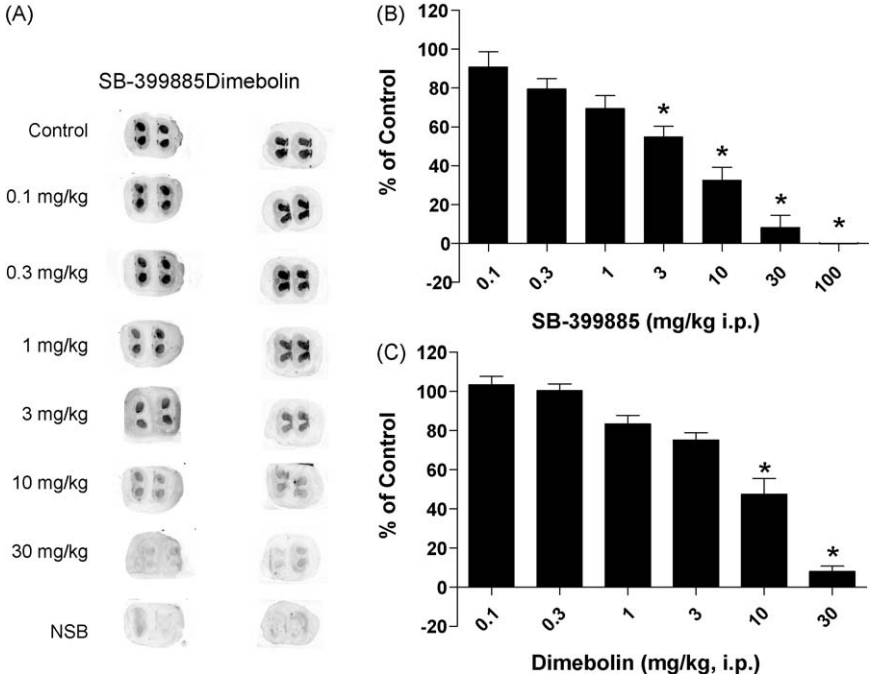

Fig. 3. Inhibition of [^{125}I]SB-258585 *ex vivo* binding in coronal rat brain slices following i.p. administration of SB-399885 and dimebolin. (A) Representative digital images obtained after a 15 h acquisition with the β-imager showing a gradual and complete inhibition of [^{125}I]SB-258585 binding with increasing dose of SB-399885 and dimebolin. SB-399885 (B), and dimebolin (C) dose-dependently inhibited [^{125}I]SB-258585 binding *ex vivo*. Data are expressed as a mean of percent of control ± S.E.M. obtained from 5 to 6 animals for each dose in three independent experiments. $^{*}p < 0.05$ vs vehicle (ANOVA, Dunnett's *post hoc* test). Average ID_{50} values ± S.E.M. are given in Table 2.

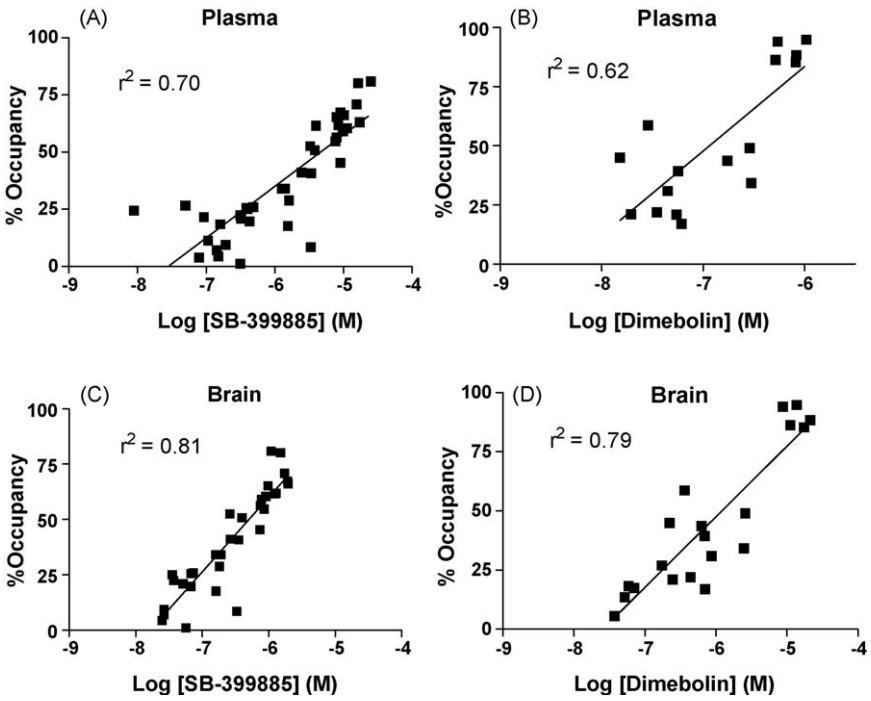

Fig. 4. Relationship between plasma and brain concentration and *ex vivo* receptor occupancy of SB-399885 and dimebolin. Data points represent measures from all animals that exhibited brain and/or plasma concentrations above the limit of detection, and measurable inhibition of [^{125}I]SB-258585 binding. Increasing plasma and brain concentrations of SB-399885 (A and C) or dimebolin (B and D) were correlated with decreased [^{125}I]SB-258585 binding. Linear regression lines and correlation coefficients are shown for each graph.

Table 2
ID_{50} and OC_{50} of SB-399885 and dimebolin measured by *ex vivo* binding.

Compound	*Ex vivo* binding ID_{50} (mg/kg i.p.)	OC_{50} (nM)
SB-399885	8.9 ± 2.5	490
Dimebolin	7.8 ± 0.5	355

ID_{50} values were obtained from Fig. 3B and C and represent the mean ± S.E.M. from 3 independent experiments. OC_{50} values represent the interpolated brain concentration producing 50% of occupancy obtained from Fig. 4C and D.

399885 did not alter investigative behavior when a novel juvenile was substituted on the second trial (Fig. 4A) suggesting that the effects of this antagonist are not due to a reduction in exploratory behavior, but instead suggest an enhanced memory for the familiar juvenile. Dimebolin also produced a reduction in RID (Fig. 5B) which reached significance at the 10 and 30 mg/kg i.p. dose (ANOVA $F_{6,121} = 13.77$; $p < 0.05$; Dunnett's *post hoc*). In contrast to the effect of SB-399885, dimebolin (30 mg/kg i.p.) significantly inhibited investigative behavior when a novel juvenile was substituted on the second trial, indicating that the effect of this compound on RID cannot be interpreted as specific to memory. Investigation of novel juveniles was not disrupted by the 10 mg/kg dose (Fig. 4B) suggesting that this dose produced an enhancement in short-term memory.

4. Discussion

Dimebolin, a non-selective antihistamine originally approved in Russia for the treatment of allergy [1] is known to have a rich pharmacological profile. The compound has been found to be neuroprotective in cellular models of AD and Huntington's disease [3,7] and to preserve learning with chronic treatment after AF64A-

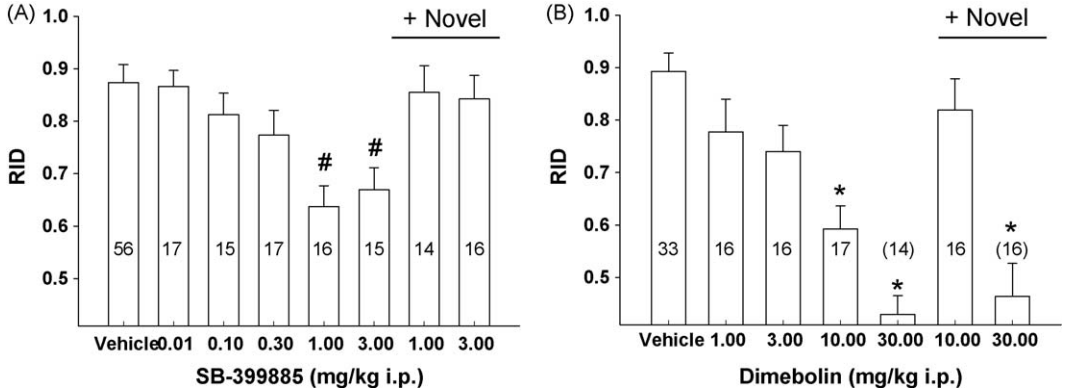

Fig. 5. Effect of SB-399885 (A) and dimebolin (B) in the rat social recognition model. Adults rats were introduced to a juvenile for an initial exposure and again after 2 h. Vehicle treated animals investigated the juvenile for the same amount of time on both occasions resulting in a Ratio of Investigation Duration (RID) near 1. Vehicle or SB-399886 or dimebolin (B) were administrated 2 h prior the second exposure. *N*-values are indicated at the base of each bar, or parenthetically above the bar. $^{\#}p < 0.01$, $^{*}p < 0.05$ vs vehicle (ANOVA, Dunnett's *post hoc* test).

induced lesion of the cholinergic system [2,4]. Interest in dimebolin has recently increased with the report of significant effects on cognitive performance in a phase II clinical trial in mild to moderate AD patients [5]. Given dimebolin's efficacy in both preclinical models and in the clinic, it is of interest to determine the molecular targets of this compound.

The known targets for dimebolin include acetylcholinesterase [2], N-methyl-D-aspartate receptors [3,6], voltage-gated calcium channels [7], and the mitochondrial permeability transition pore [8]. In addition, dimebolin at a 10 μM concentration has been reported to inhibit binding to a number of targets including α-adrenergic, dopamine, and serotonin receptors, including the h5-HT_6 receptor [3]. The present findings confirm the binding activity of dimebolin at the h5-HT_6 receptor, and demonstrate binding to and functional antagonism of both the rat and human 5-HT_6 receptor. The affinity and potency of dimebolin for recombinant human and rat 5-HT_6 receptor and the native r5-HT_6 receptor are lower than that observed with the selective 5-HT_6 antagonist SB-399885. This is not unexpected as SB-399886 has been optimized for this activity. While the potency of dimebolin for the 5-HT_6 receptor is approximately 100-fold lower than SB-399885, it is in the nanomolar range, which is in stark contrast to potencies reported to date for other targets of dimebolin, all of which are in the 5–50 μM range [2,3]. Interestingly, despite the difference in 5-HT_6 affinity, both dimebolin and SB-399885 occupy the 5-HT_6 receptor *in vivo* based on the *ex vivo* estimates in the current study, and reach 50% occupancy at similar doses and at similar brain concentrations. This is likely due to the fact that *in vivo* occupancy is a complex function of a number of variables other than binding affinity including bioavailability, brain penetrance, plasma protein binding, and compound free fraction.

As explained in Section 2, the striatum was chosen as the region of interest for the quantification of 5-HT_6 receptor occupancy based on practical considerations. However, it is notable that 5-HT_6 receptors have been implicated in the modulatory control of striatal GABA and acetylcholine tone [28]. While the effect of 5-HT_6 receptor antagonism in the social recognition test of short-term memory employed in the present study is likely mediated at the level of the cortex or hippocampus [29] interestingly, the striatum plays an important role in other forms of cognitive function such as instrumental learning, a behavior that is impaired by over expression of 5-HT_6 receptors in the striatum [30]. The presence of 5-HT_6 receptors in the striatum, and the actions of dimebolin at this site may be indicative of potential targets for cognitive dysfunction in disorders with significant striatal involvement such as Parkinson's and Huntington's disease.

The finding that dimebolin improves short-term social recognition memory in normal rats expands upon previous studies in cholinergically denervated animals [2,4] and suggests that the pro-cognitive effects of this compound can be acute and will likely generalize to disorders that do not involve disruption in cholinergic function. While it is tempting to interpret the effect of dimebolin on short-term memory as being linked to the antagonism of the 5-HT_6 receptor, there are differences between the effect of dimebolin and the selective 5-HT_6 antagonist SB-399885 that should be noted. Despite similar *ex vivo* ID_{50} and OC_{50} values for dimebolin and SB-399885, the later compound is 10-fold more potent at enhancing short-term memory in the social recognition test. The only other studies to examine the effect of dimebolin on cognitive function employed a 1 mg/kg i.p. dose administered chronically for 12–14 days prior to testing [2,4]. Since the prior studies did not assess the acute effects of dimebolin, it is impossible to draw a comparison between these studies and the efficacious dose in the present studies. As noted, dimebolin binds to a number of identified targets with micromolar affinity, and may bind to other unidentified targets. Therefore, the lower potency of dimebolin in the social recognition assay compared to SB-399885 may be due to interactions with other targets that counteract the effect of 5-HT_6 antagonism.

Consistent with the suggestion that other activities of dimebolin act to partially counteract the effect of 5-HT_6 is the finding that the highest dose of dimebolin tested in these studies (30 mg/kg i.p.) produced a disruption in normal exploratory behavior. This may be due to the higher doses of dimebolin leading to brain concentrations capable of fully engaging other targets. Interestingly dimebolin has been shown to block NMDA-induced seizures with an ED_{50} of 42 mg/kg i.p., suggesting that the 30 mg/kg i.p. dose used in the present study may produce significant block of central NMDA receptors [2]. As the NMDA receptor is involved in a number of relevant behaviors including learning and locomotion, this may contribute the effect of dimebolin on normal exploration. Importantly, the animals treated with 30 mg/kg i.p. dimebolin appeared relatively normal with the exception of a slight decrease in activity. It should be stressed that the lack of social exploratory behavior only indicates that the effect of the compound on recognition of the familiar juvenile at this dose cannot be ascribed to enhanced cognitive function. This finding cannot be interpreted to suggest that there is an inhibition of cognitive function.

While an old compound, dimebolin potentially represents a novel breakthrough in the treatment of AD. The present study characterizes the 5-HT_6 receptor as an additional target underlying the efficacy of dimebolin, and demonstrates an acute enhancement of cognitive function. It is important to note that dimebolin interacts with at least 17 additional targets [3] and that these interactions have not been fully characterized. Since many of these targets could also impact on cognitive function, the relative role of 5-HT_6 receptor antagonism in the clinical efficacy of dimebolin remains speculative. Future studies employing additional selective pharmacological tools, or perhaps genetically modified animals lacking the 5-HT_6 receptor, will be required to make a direct link between this acute effect of dimebolin on cognition and the compound's activity at the 5-HT_6 receptor. The rich pharmacological profile of dimebolin is reminiscent of other neuroactive drugs such as clozapine and its apparent efficacy in AD patients reinforces the view that complex disorders may respond best to pharmacologically complex compounds rather than highly specific drugs directed against a single molecular target [31].

Acknowledgements

We thank Beverly Holskin, Sheryl Meyer, Kristen Murray and Chrysanthe Spais for engineering cell lines and generation of cell membranes, Thelma Angeles, Emir Duzic, Karla Kopec and Rita Raddatz for their technical assistance in conducting the pharmacological characterization in recombinant cell lines, Rebecca Brown and Deborah Galinis for conducting compound quantification studies and Robert Bendesky for conducting social recognition studies.

This study was supported by Cephalon Inc.

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