Cognition-Enhancing Properties of Dimebon in a Rat Novel Object Recognition Task Are Unlikely to Be Associated with Acetylcholinesterase Inhibition or N-Methyl-D-aspartate Receptor Antagonism

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ABSTRACT

Dimebon (dimebolin) treatment enhances cognition in patients with Alzheimer’s disease (AD) or Huntington’s disease. Although Dimebon was originally thought to improve cognition and memory through inhibition of acetylcholinesterase (AChE) and the N-methyl-D-aspartate (NMDA) receptor, the low in vitro affinity for these targets suggests that these mechanisms may not contribute to its clinical effects. To test this hypothesis, we assessed whether Dimebon enhances cognition in rats and if such an action is related to either mechanism or additional candidate mechanisms. Acute oral administration of Dimebon to rats (0.05, 0.5, and 5 mg/kg) enhanced cognition in a novel object recognition task and produced Dimebon brain concentrations of 1.7 ± 0.43, 14 ± 5.1, and 172 ± 94 nM, respectively. At these concentrations, Dimebon did not alter the activity of recombinant human or rat brain AChE. Unlike the AChE inhibitors donepezil and galantamine, Dimebon did not change acetylcholine levels in the hippocampus or prefrontal cortex of freely moving rats. Dimebon displays affinity for the NMDA receptor (Kᵢ = 105 ± 18 μM) that is considerably higher than brain concentrations associated with cognition enhancement in the novel object recognition task and 200-fold weaker than that of memantine (Kᵢ = 0.54 ± 0.05 μM). Dimebon did not block NMDA-induced calcium influx in primary neuronal cells (IC₅₀ > 50 μM), consistent with a lack of significant effect on this pathway. The cognition-enhancing effects of Dimebon are unlikely to be mediated by AChE inhibition or NMDA receptor antagonism, and its mechanism of action appears to be distinct from currently approved medications for AD.

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Dimebon (latrepirdine, dimebolin) is an investigational drug in Phase 3 clinical trials for the treatment of Alzheimer’s disease (AD) and Huntington’s disease (HD). Dimebon improved cognitive function in patients with AD during a 6-month placebo-controlled study with a 6-month open-label extension (Doody et al., 2008), and patients with HD showed some benefits in cognition in a 3-month Phase 2 study (Kieburtz et al., 2010). In the AD trial, patients treated with Dimebon also showed improvement over placebo in activities of daily living and in the behavioral and neuropsychiatric symptoms of AD, suggesting broad-based clinical improvement (Doody et al., 2008). The mechanism by which Dimebon exerts the favorable effects reported in these clinical studies is not clearly understood. Current AD therapeutics fall into two main pharmacologic classes: acetylcholinesterase (AChE) inhibitors, including donepezil, galantamine, and rivastigmine (Birks, 2006;
Raina et al., 2008), and N-methyl-D-aspartate (NMDA) receptor antagonists, of which memantine is the only approved agent (McShane et al., 2006; Peskind et al., 2006; Raina et al., 2008). The pharmacologic basis for the clinical benefits of Dimebon in AD and HD patients is unclear. Early studies suggested that Dimebon inhibits AChE (IC50 = 42 μM) and prevents NMDA-induced seizures (EC50 = 4 mg/kg), although at relatively high concentrations or doses (Bachurin et al., 2001).

The present studies were performed to determine whether the cognition-enhancing properties of Dimebon in rodent models may be the result of activity at these two targets. Because AChE inhibitors increase acetylcholine (ACh) levels in the brain by inhibiting its degradation, we tested whether Dimebon can modulate this neurotransmitter in the rat brain at doses effective for enhancing cognition. We also determined whether NMDA receptor antagonism or interactions with other neurotransmitter receptors might play a role in mediating the cognitive effects of Dimebon in rodent models and at doses of Dimebon compatible with blood levels attained during cognitive enhancement in the rat novel object recognition task.

Materials and Methods

Drugs. 2,8-Dimethyl-5-[2-(6-methyl-3-pyridyl)ethyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (Dimebon dihydrochloride; provided by Medivation, Inc., San Francisco, CA) had greater than 95% purity, as determined by high-performance liquid chromatography (HPLC). Dimebon was dissolved in water for oral gavage, and solutions were prepared fresh each day and administered orally in a volume of 10 ml/kg. Donepezil hydrochloride (Sequoia Research Products, Pego- bourne, UK) and galantamine hydrobromide (Tocris Bioscience, Ellisville, MO) were dissolved in sterile saline and administered intraperitoneally (donepezil) in a volume of 5 ml/kg and subcutaneously (galantamine) in a volume of 1 ml/kg.

Novel Object Recognition Task. Experimental animals. Male Sprague-Dawley rats (Centre d’Elevage R., Janvier, France) weighing between 230 and 300 g and 6 to 8 weeks of age were used. Animals were housed in groups of two to four on a 12-h on/12-h off light cycle and had ad libitum access to food and water. All aspects of animal housing and handling were conducted under Accreditation of Laboratory Animal Care and provisions of the Biotrial Institutional Animal Care and Use Committee.

Behavioral paradigm. The procedures used for the novel object recognition (NOR) task have been described previously (Bertaina-Anglade et al., 2007). The rats were acclimated to the arena (hollow cube 60 × 60 × 40 cm) without objects for 30 to 45 min before testing. The NOR consisted of two trial periods (T1 and T2) separated by a 24-h intertrial period. Rats were dosed orally with vehicle (water) or Dimebon (0.05, 0.5, or 5 mg/kg) or intraperitoneally with the positive control donepezil (1 mg/kg) 30 min before the acquisition trial (T1) and then placed in the arena containing two identical objects. The time required for each animal to complete 15 s of total exploration of the two identical objects, as shown by placing its nose within 2 cm of the object, was determined, with a cutoff of 240 s. Locomotor activity (expressed as the number of lines crossed on the arena’s floor) was also scored during T1 and T2. For the retention trial (T2) conducted 24 h later, one of the objects presented in T1 was replaced with a novel object. Rats were returned to the arena for 3 min, and the duration of exploration of each object was scored. All the measurements were made by a trained observer blind to the experimental treatments. A criterion of minimal level of object exploration was used to exclude animals with low levels of spontaneous exploration; thus, only animals having a minimal level of object exploration of ≅5 s during the retention trial T2 (novel + familiar ≅5 s) were included.

Based on this criterion, two animals were excluded in the 0.5 mg/kg Dimebon group and one animal in the 5 mg/kg Dimebon group.

Statistical analysis. Statistical analysis was performed using SAS software (SAS for Windows, version 8.2; SAS Institute, Cary, NC). The difference between time spent exploring the novel object versus time spent exploring the familiar object during T2 was analyzed for each independent treatment group using a two-sided Student’s t test for paired samples. The level of significance was set at α = 0.05. In addition to a one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test was used to compare the effect of Dimebon or donepezil versus vehicle treatments on the absolute difference between time spent exploring familiar and novel objects. For analysis of time required to achieve 15 s of object exploration during T1 and for locomotor activity during T1 or during T2, a two-sided Student’s t test for independent samples was used to compare vehicle versus donepezil. Comparison of vehicle versus Dimebon groups was performed using a one-way ANOVA.

Evaluation of Dimebon Concentrations in Rat Brain and Plasma. To assess brain and plasma exposures of Dimebon over time, adult male Sprague-Dawley rats (250–300 g b.w.; n = 3 per time point) were dosed by oral gavage with 0.05 mg/kg Dimebon in water. Venous blood and brain samples were obtained at 15 and 30 min and 1, 2, 4, and 6 h and processed as described below.

A second group of adult male Sprague-Dawley rats (n = 4/group) was treated identically to the rats receiving Dimebon 0.05, 0.5, and 5 mg/kg p.o. in the NOR. Rats were sacrificed under pentobarbital (60 mg/kg) anesthesia ~50 min after dosing. Venous blood was obtained from the vena cava and placed in prechilled 2-ml K2 EDTA collection tubes and centrifuged within 15 min at 2000 g for 10 min at 4°C. Brains from these animals were midcoronally bisected, and one hemisphere from each rat was prepared for Dimebon content analysis.

Dimebon and the internal standard, N-13C deuterated Dimebon, were isolated from plasma and whole brain homogenates by methanol-induced protein precipitation. After centrifugation, supernatant fractions were analyzed by reverse-phase HPLC. A solvent gradient was used for separation, and the effluent was directed to a tandem mass spectrometry (MS) system equipped with electrospray ionization source. Positive ions were detected in the multiple reaction monitoring mode with precursor → product ion pairs of 320.2 → 277.2 for Dimebon and 324.3 → 277.2 for the internal standard, N-13C deuterated Dimebon. Assays were calibrated using standard curves from nine duplicate calibration standards, which yielded a linear response–concentration curve having a coefficient of determination >0.98. Precision and accuracy, which were measured through “quality control” samples, were within 15%. The lower limits of quantification were 1 pg/ml in plasma and 1 pg/g in brain.

Effects on AChE. Evaluations of the inhibitory potency of Dimebon and donepezil for AChE were made with three different in vitro methods. Using recombinant human embryonic kidney 293 cell-derived human AChE enzyme, inhibitory potency was determined by the inhibition of the conversion of the AChE substrate acetylthiocholine-iodide to thiocholine (Ellman et al., 1961; Nadarajah, 1992) (MDS Pharma Services, Taipei, Taiwan). AChE inhibitory activity of Dimebon (0.08–31,000 nM) was determined ex vivo with freshly prepared human red blood cell fractions and rat brain extracts (Ellman et al., 1961; Nadarajah, 1992) using donepezil (0.005–500 nM) and eserine as positive controls. Nonlinear regression analyses were used to calculate the IC50 values for AChE inhibition by Dimebon and donepezil.

In Vivo Microdialysis. Experimental animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing between 280 and 350 g were used. Animals were housed in groups of two to four on a 12-h on/12-h off light cycle and had ad libitum access to food and water. All aspects of animal housing and handling were conducted in accordance with the Brains On-Line Institutional Animal Care and Use Committee guidelines.
Experimental procedures. Based on established microdialysis procedures (Cremers et al., 2009), rats were anesthetized with isoflurane (2%, 800 ml/min O2) and placed in a stereotactic frame (Kopf Instruments, Tujunga, CA). One L-shaped probe with 4 mm of exposed surface (Hospal AN 69 membrane; Brainlink, Groningen, The Netherlands) was inserted into the prefrontal cortex at the following coordinates: posterior −3.4 mm from bregma, lateral −0.9 mm to midline, and ventral −5.0 mm to dura, or into the ventral hippocampus: posterior −5.3 mm to bregma, lateral −4.8 mm to midline, and ventral −8.0 mm to dura (Paxinos and Watson, 1998). Within 24 to 48 h from surgery, the microdialysis probe was connected with flexible plastic tubing to a microperfusion pump (Syringe pump PHD 2000; Harvard Apparatus Inc., Holliston, MA) and perfused at a constant rate of 1.5 μl/min with artificial cerebrospinal fluid (ACSF), containing 147 mM NaCl, 3 mM KCl, 1.2 mM CaCl2, and 1.2 mM MgCl2. Microdialysis samples (30 μl each) were collected at 20-min intervals with an automated refrigerated fraction collector (820-20 Univentor microsampler; Univentor, Zejtun, Malta) into minivials containing 20 μl of 0.02 M formic acid and were stored at −80°C. After collection of four basal samples, animals were treated with vehicle (water), Dimebon (0.05, 0.5, and 5 mg/kg p.o.), and either the positive control galantamine (0.03 mg/kg s.c.) for hippocampal studies or donepezil (1 mg/kg i.p.) for prefrontal cortex studies. Samples were collected for 3.5 h after dosing.

Analysis of ACh content in microdialysis samples. Microdialysis samples were mixed with 10 μl of internal standard (acetyl-β-methylcholine; Sigma-Aldrich, St. Louis, MO) and injected into an HPLC/MS/MS system by an automated sample injector (SIL-20 AChT, Shima, Kyoto, Japan). Chromatographic separations of the internal standard and ACh were performed by a 150 × 2.1-mm, 5-μm ion exchange analytical column (BioBasic SCX; Thermo Fisher Scientific, Waltham, MA) at 30°C. The mobile phase was composed of 15 mM ammonium acetate and 10 mM ammonium formate (pH 4.0; flow rate, 0.3 ml/min) and a linear acetonitrile gradient to 20, 80, and 20% was created for each sample from 0 to 2, 2 to 4.2, and 4.2 to 5 min postinjection, respectively. The HPLC effluent containing ACh and the internal standard was directed to the MS/MS detector from 2.3 to 3.5 min of solvent elution. Compound analysis was performed by an API 3000 MS/MS detector and a Turbo Ion Spray interface (both from MDS Sciex, Concord, ON, Canada). Acquisitions were performed in positive ionization mode, with ion spray voltage at 4.5 kV with a probe temperature of 450°C. The instrument was operated in multiple reaction monitoring mode. Data were calibrated and quantified using the Analyst data system (version 1.4.2; Applied Biosystems, Foster City, CA). Mass transitions for acetyl-β-methylcholine were 146.1 and 160.0 at Q1 and 87.0 and 101.0 at Q3.

Statistical analysis. The femtomole perfusate values of ACh for different concentrations of Dimebon (0, 0.05, 0.5, 5.0, and 50 μM) or memantine (0, 5, 10, 25, and 50 μM). Fluo-3 fluorescence was quantified with the Synergy 4 hybridized modality using 485/20 nm excitation and 530/25 nm emission bandwidth filters. Data were collected at 24-s intervals. The volume in each well was 100 μl, and 100 μl of an equivalent solution containing Dimebon 2× (20 μM) was applied to the corresponding well using a multichannel pipette. Control experiments, where ACSF with no drug/NMDA was added, showed that cells reach a stationary fluorescence signal by −5 min after the volume addition. All of the experiments were performed at 37°C. Fluorescence values (Ft) were normalized against initial values (F0) before NMDA addition using the formula (Ft − F0)/F0.

In Vitro Assays for Dimebon Binding to Other Neurotransmitter Receptors and Functional Assessment for the Histamine H1 Receptor. The binding of Dimebon to a broad set of additional neurotransmitter receptors including those implicated with cognition was conducted by high-throughput screening (MDS Pharma Services). Binding was first determined at a Dimebon concentration of 10 μM, and IC50 values were calculated for each receptor that was occupied at least 50% by 1 μM Dimebon.

The potential histamine H1 receptor agonist and antagonist properties of Dimebon were evaluated using an in vitro guanosine 5′-3′-O-(thio)triphosphate (GTPγS) binding assay (De Backer et al., 1993). In brief, samples containing Dimebon or the H1 antagonist pyrilamine were preincubated for 30 min with a membrane fraction from Chinese hamster ovary-K1 cells that were engineered to express the human H1 receptor. The reaction was initiated with 0.3 nM guanosine 5′-O-(3′-[35S]S)triphosphate for an additional 30 min and with 0 or 1000 nM histamine to assess agonist or antagonist properties of Dimebon, respectively. Bound [35S] was determined as described by De Backer et al. (1993).

Results

Cognitive-Enhancing Effects of Dimebon. The acute cognition-enhancing effects of Dimebon were shown by its ability to delay natural forgetting in rats after a 24-h intertrial interval in the NOR task. Rats acutely treated with Dimebon at 0.05, 0.5, or 5 mg/kg 30 min before T1 explored the novel object significantly more than the familiar one during T2 (p = 0.0085, p = 0.0013, and p < 0.0001, respectively), showing an increase in memory retention. These effects of acute Dimebon administration were qualitatively similar to those obtained with the positive control donepezil (1 mg/kg i.p.) (Fig. 1A). The absolute difference between time spent with novel
and familiar objects was 0.8 ± 0.9, 4.3 ± 1.4, 4.9 ± 1.2, 6.2 ± 1.1, and 6.1 ± 1.3 s for the vehicle, 0.05 mg/kg Dimebon, 0.5 mg/kg Dimebon, 5 mg/kg Dimebon, and donepezil groups, respectively (*, p < 0.05 for comparisons with the vehicle group by one-way ANOVA followed by Dunnett's post hoc test).

In addition, the time required to achieve 15 s of object exploration during T1 was not affected by acute Dimebon administration [F(3,45) = 1.13; p = 0.349] (Fig. 1B). Furthermore, Dimebon did not alter locomotor activity during T1 [F(3,45) = 0.28; p = 0.838] (Fig. 1C) or T2 [F(3,45) = 0.92; p = 0.441] (Fig. 1D) compared with values for the vehicle-treated animals. Likewise, donepezil did not change the time required to achieve 15 s of object exploration during T1 (p = 0.0618), neither did it affect locomotor activity during T1 (p = 0.461) (Fig. 1C) nor T2 (p = 0.385) (Fig. 1D).

Brain concentrations of Dimebon associated with cognitive enhancement in the NOR task were determined in a pharmacokinetic study after a 0.05 mg/kg oral dose. The plasma C<sub>max</sub> occurred at 0.25 h, indicating rapid oral absorption. Brain penetration was also rapid as indicated by the finding that brain tissue concentrations exceeded those of plasma at all the sample times. The brain C<sub>max</sub> occurred at 1 h; subsequent to this peak, the brain and plasma concentration-time curves were essentially parallel (Fig. 2), and the mean brain/plasma concentration ratio ranged from 9.0 to 10.8. When rats were orally administered the same doses of Dimebon used in the NOR task (0.05, 0.5, and 5.0 mg/kg), increases in Dimebon concentrations in brain and plasma (Table 1) were observed at approximately 50 min after dosing. Brain concentrations resulting from these pharmacologically effective doses ranged from 1.7 to 172 nM after oral administration of 0.05 and 5 mg/kg doses, respectively.

**Effect of Dimebon on AChE Activity.** Dimebon was consistently shown to be a weak inhibitor of AChE using three separate assays. AChE inhibition was evaluated in vitro with a recombinant human enzyme preparation. Donepezil inhibited recombinant human AChE with an IC<sub>50</sub> value.
of 0.028 ± 0.005 μM (mean ± S.D.), whereas Dimebon was nearly 3000-fold less potent (IC₅₀ value of 83 ± 13 μM; n = 3 determinations for each compound) (Fig. 3; Table 2). To assess whether Dimebon had a more potent effect on a natural human form of AChE, inhibitory activity in a red blood cell fraction was tested with Dimebon and donepezil. The IC₅₀ of Dimebon for inhibiting AChE activity was >31 μM, or at least 3000-fold less potent than donepezil, which inhibited AChE activity with an IC₅₀ value of 0.010 ± 0.003 μM (Fig. 4; Table 2). Finally, to assess whether Dimebon was more effective at inhibiting the AChE enzyme as it occurs in the brain, a rat brain fraction containing AChE was used as a source of enzyme. Dimebon at doses up to and including 31 μM did not have a significant effect on AChE activity from rat brain fraction, whereas donepezil was effective with an IC₅₀ of 0.012 μM (Fig. 5; Table 2).

**Extracellular ACh Levels in the Rat Brain after Acute Dimebon Administration.** To assess further whether Dimebon modulates AChE activity or other pathways that regulate extracellular ACh levels in the brain, in vivo microdialysis was performed in rat hippocampus and prefrontal cortex regions to measure this neurotransmitter using doses of Dimebon (0.05, 0.5, or 5 mg/kg) that displayed cognitive enhancement in the NOR task. Acute administration of different doses of Dimebon did not significantly alter extracellular levels of ACh in either the hippocampus [F(3,0,294) = 1.47; p = 0.06] (Fig. 6A) or the prefrontal cortex [F(3,0,275) = 1.15; p = 0.27] (Fig. 6B) of freely moving rats. A two-way ANOVA indicated a significant time × treatment interaction for the effect of 0.6 mg/kg galantamine [F(10,116) = 7.65; p < 0.001] on hippocampal ACh levels with a maximal increase of approximately 350% over basal levels. In addition, a two-way ANOVA revealed a significant time × treatment interaction for the effect of 1 mg/kg donepezil [F(10,145) = 5.305; p < 0.001] on extracellular prefrontal cortex ACh levels with a maximal increase of approximately 500% over basal levels.

**Interaction with the NMDA Receptor.** Cell binding and a cell-based assay of NMDA-induced calcium internalization measured by the fluorescent calcium indicator fluo-3 were used to compare Dimebon versus memantine interactions with the NMDA receptor. Dimebon (mean ± S.D., Kᵢ = 105 ± 18 μM; n = 3 per Kᵢ determination) was nearly 200-fold less potent than memantine (Kᵢ = 0.54 ± 0.05 μM) in occupying the phencyclidine binding site of the NMDA receptor (Fig. 7) and was ineffective at blocking NMDA-induced Ca²⁺ influx (IC₅₀ > 50 μM) (Fig. 8A). In contrast, memantine showed moderately potent blockade of NMDA-induced Ca²⁺ influx (Fig. 8B). The IC₅₀ for memantine was calculated as 1.89 ± 3.23 μM (mean ± S.D.; n = 3).

**Interactions with Other Cognition-Related Targets.** The ability of Dimebon to bind to neurotransmitter and neuromodulatory binding sites (61), ion channels (7), neurotransmitter transporters (5), and other targets (11) was evaluated by in vitro studies (see Supplemental Table). Neurotransmitter receptors for which higher affinity binding of Dimebon were obtained (Kᵢ values ≤ 900 nM) are listed in Table 3. Consistent with its historical use as an antihistamine, Dimebon displayed the highest affinity for binding to the histamine H₁ receptor (Kᵢ = 1.3 nM). Because agonists of the histamine H₁ receptor have been implicated in memory, the agonist and...
antagonist properties of Dimebon were determined with an in vitro GTP\(\gamma\)S binding assay (De Backer et al., 1993) using a cell line engineered to express the human H\(_1\) receptor. Consistent with an antagonist profile, Dimebon showed a concentration-dependent inhibition of histamine-induced GTP\(\gamma\)S binding with an IC\(_{50}\) of 76.5 nM. Pyrilamine, the positive antagonist control, had an IC\(_{50}\) of 51.5 nM. No agonist properties were seen with Dimebon concentrations up to 5000 nM (data not shown). Dimebon was found to bind to several other neurotransmitter receptors with high affinity, including those for serotonin, norepinephrine, dopamine, and imidazolines (Table 3). Dimebon at 3 \(\mu\)M did not bind with several neurotransmitter receptors implicated in memory, including muscarinic and nicotinic ACh receptors and the histamine H\(_3\) receptor (Table 4).

**Discussion**

The present findings suggest that Dimebon's cognition-enhancing effects in the rat NOR task are unlikely to be mediated by inhibition of AChE or via antagonism of the NMDA receptor. To better understand the pharmacology of Dimebon, its effect on cognition was evaluated in the rat

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**Fig. 4.** Effect of Dimebon and donepezil on AChE activity in freshly prepared human red blood cell extract. Dimebon (A) or donepezil (B) was added to a red blood cell fraction freshly prepared from three volunteers, and AChE activity was measured with the acetylthiocholine-iodide substrate method (Ellman et al., 1961). Values are given as mean ± S.D., with assays performed in triplicate.

**Fig. 5.** Effect of Dimebon and donepezil on AChE activity in freshly prepared rat brain extract. Dimebon or donepezil was added to a rat brain extract, and AChE activity was measured using the acetylthiocholine-iodide substrate method (Ellman et al., 1961). Values are means, with assays performed in triplicate.
NOR model. This assay is sensitive to a variety of pharmacologic agents that act through different brain pathways (Dere et al., 2007). Although the NOR task model in healthy rodents is not a model for AD or HD, the pathway for enhancing cognition that is modulated by Dimebon may be functioning in patients with AD or HD, and thus can be used for some mechanistic studies. Dimebon enhanced cognition in this rodent model of short-term memory at acute oral doses of 0.05, 0.5, and 5.0 mg/kg. Dimebon concentrations in the brain are important for considering candidate mechanisms for its cognitive-enhancing effects in rats. Although metabolism of Dimebon occurs after oral dosing of rats, the parent molecule is the predominant species in the brain (data not shown). Brain exposure associated with the lowest pharmacologically effective dose of Dimebon (0.05 mg/kg) was 1.7 nM, and higher doses were associated with roughly proportionately higher exposures. It is not known how much of the drug is unbound to nonspecific proteins, carbohydrates, and lipids, and thus available for pharmacologic effects; consequently,

Fig. 6. Extracellular ACh in the hippocampus (A) and prefrontal cortex (B) of freely moving rats after administration of vehicle (water), Dimebon (0.05, 0.5, and 5 mg/kg p.o.), or AChE inhibitors galantamine (0.63 mg/kg s.c.) or donepezil (1 mg/kg i.p.). The arrow indicates the time when the test compound or vehicle was administered. Values are mean ± S.E.M., n = 8/group.

Fig. 7. Effects of Dimebon and memantine on the NMDA receptor; binding was determined by radioligand binding to the phencyclidine binding site of the NMDA receptor (Goldman et al., 1985), n = 3/Kᵢ determination.

Fig. 8. Intracellular NMDA-dependent calcium levels in primary rat neurons with Dimebon (0.05, 0.5, 5.0, 50 μM) (A) and memantine (5, 10, 25, 50 μM) (B) treatment. Control was water. Traces shown are the mean ± S.E.M. results of the fluorescence obtained by four replicates per treatment 5 min before and 5 min after NMDA addition.
TABLE 3
Receptors to which the affinity ($K_i$) of Dimebon binding was ≤900 nM
A panel of additional receptor or enzyme targets showed interactions with Dimebon that were considerably weaker than what is shown here ($K_i > 1$ mM; see Supplemental Table.

<table>
<thead>
<tr>
<th>Target</th>
<th>Receptor Species and Source</th>
<th>Competing Ligand</th>
<th>$K_i$ Mean ± S.D.$^a$</th>
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<tr>
<td>Histamine, H₁</td>
<td>Human recombinant in CHO-K1 cells</td>
<td>Pyrillamine</td>
<td>1.3 ± 0.3</td>
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<tr>
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<td>Human recombinant in CHO cells</td>
<td>Lysergic acid diethylamide</td>
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<tr>
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<td>Rauwolscine</td>
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<tr>
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<td>Prazosin</td>
<td>21.4 ± 7.0</td>
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<tr>
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<td>Prazosin</td>
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<tr>
<td>Serotonin, 5-HT₁₈</td>
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<td>Lysergic acid diethylamide</td>
<td>42.2 ± 7.2</td>
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<td>Adrenergic, α₁C</td>
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<td>MK912</td>
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<td>Adrenergic, α₂A</td>
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<td>MK912</td>
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<td>Imidazoline, I₂ (central)</td>
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<td>Idazoxan</td>
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<tr>
<td>Histamine, H₂</td>
<td>Human recombinant CHO-K1 cells</td>
<td>Aminopentidine</td>
<td>201 ± 115</td>
</tr>
<tr>
<td>Dopamine, D₁</td>
<td>Human recombinant CHO cells</td>
<td>S-Neurolept</td>
<td>339 ± 211</td>
</tr>
<tr>
<td>Dopamine, D₂</td>
<td>Human recombinant CHO cells</td>
<td>Spiperone</td>
<td>539 ± 339</td>
</tr>
<tr>
<td>Dopamine, D₃</td>
<td>Human recombinant CHO cells</td>
<td>SCH23390</td>
<td>683 ± 429</td>
</tr>
<tr>
<td>Serotonin, 5-HT₂B</td>
<td>Human recombinant CHO-K1 cells</td>
<td>Lysergic acid diethylamide</td>
<td>875 ± 700</td>
</tr>
</tbody>
</table>

CHO, Chinese hamster ovary; HEK, human embryonic kidney.

$^a$ All the assays performed with $n = 3$.

TABLE 4
Low affinity of Dimebon for select receptors implicated in cognition

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Receptor Species and Source</th>
<th>Competing Ligand</th>
<th>$IC_{50}$ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscarinic acetylcholine receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₁</td>
<td>Human recombinant CHO cells</td>
<td>Methylscopolamine</td>
<td>&gt;10</td>
</tr>
<tr>
<td>M₂</td>
<td>Human recombinant CHO cells</td>
<td>Methylscopolamine</td>
<td>&gt;10</td>
</tr>
<tr>
<td>M₄</td>
<td>Human recombinant CHO cells</td>
<td>Methylscopolamine</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Nicotinic acetylcholine receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α₇</td>
<td>Human RD cells</td>
<td>α-Bungarotoxin</td>
<td>&gt;3</td>
</tr>
<tr>
<td>α₇β₂</td>
<td>Rat brain</td>
<td>Cytisine</td>
<td>&gt;3</td>
</tr>
<tr>
<td>α₇</td>
<td>Rat brain</td>
<td>α-Bungarotoxin</td>
<td>&gt;3</td>
</tr>
<tr>
<td>α₇</td>
<td>Rat brain</td>
<td>Methyllycaconitine</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Histamine receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂</td>
<td>Human recombinant CHO-K1 cells</td>
<td>Methylhistamine</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

CHO, Chinese hamster ovary.

this concentration of Dimebon may be an overestimation of exposure required for activity. Nevertheless, the low nanomolar brain concentrations provide a reference point for considering candidate mechanisms for Dimebon’s cognition-enhancing effects in rats.

Dimebon’s inhibitory potency on recombinant human AChE was determined to be 3000-fold less than donepezil and considerably weaker than what would be required for cognitive enhancement in rats where brain exposures are as low as 1.7 nM. The lack of significant AChE inhibitory effects with preparations from human red blood cells and rat brain extracts ($IC_{50}$ values > 30 µM) is consistent with the high $K_i$ value derived with the recombinant human enzyme. Similar weak inhibitory potencies against AChE were presented by Bachurin et al. (2001) ($IC_{50}$ = 42 µM), although the source of the enzyme was not reported.

Other data reported here support the conclusion that Dimebon does not significantly influence ACh turnover. For example, Dimebon doses associated with cognition enhancement in the NOR task did not alter extracellular levels of ACh in the hippocampus or prefrontal cortex, consistent with a lack of effect on AChE activity, ACh release, or synaptic turnover of ACh.

The lack of effect of Dimebon on AChE activity, as well as its 10-fold lower concentrations in plasma than brain, is also consistent with the low incidence of gastrointestinal side effects in patients with AD (Doody et al., 2008) and HD (Kieburz et al., 2010). These clinical and preclinical findings suggest that little or no peripheral cholinergic perturbations may be expected with Dimebon. In contrast, observations made during clinical trials of rivastigmine (Rösler et al., 1999), donepezil (Pratt et al., 2002), and galantamine (Wilcock et al., 2000) indicate elevated parasympathetic tone, probably as a result of enhanced peripheral cholinergic transmission in patients taking these agents.

Dimebon was 200-fold less potent at inhibiting binding to the phencyclidine binding site on the NMDA receptor than memantine and was ineffective at blocking NMDA-induced calcium influx. An antagonism of glutamate-induced calcium signals was observed by Wu et al. (2008) but only at 50 µM concentrations of Dimebon and not at lower concentrations. Thus, the cognition-enhancing properties of Dimebon are unlikely to be mediated by an antagonist action at the NMDA receptor.

Although the data presented here suggest that inhibition of AChE and antagonism at the NMDA receptor are unlikely mechanisms for the cognition enhancement produced by Dimebon, other neurotransmitter receptors for which Dime-
bon has affinity may be important. Recently Wu et al. (2008) showed that Dimebon binds to numerous neurotransmitter receptors (e.g., histamine, dopamine, norepinephrine, and serotonin) when tested at 10 μM with moderate to high affinity. The exposure data reported here suggest that concentrations of Dimebon in the brain resulting from cognition-enhancing doses are sufficient to affect some of these receptors, particularly at the higher doses; therefore, Dimebon’s cognition-enhancing effects may in part be mediated by blockade of some of these neurotransmitter receptors. However, brain concentrations associated with cognition enhancement at lower doses (e.g., 1.7 and 14 nM after 0.05- and 0.5 mg/kg doses, respectively) are well below the $K_I$ for many receptors previously associated with cognitive function.

Dimebon binds with high affinity to the histamine $H_1$ receptor with antagonist but not with agonist properties, consistent with its early use as an antiallergy medication. Preclinical and clinical studies suggest that antagonists of the histamine $H_1$ receptor have a neutral to negative effect on memory (Brewer et al., 1993; Oken et al., 1994; Simons et al., 1996; Higuchi et al., 2000; Simons, 2004; van Ruitenbeek et al., 2008; Yanai et al., 2008). In AD, several studies suggest degeneration of histaminergic neurons (Nakamura et al., 1993), reduced levels of histamine (Mazurkiewicz-Kwielecki and Nsonwah, 1989), and reduced histamine $H_1$ receptor occupancy (Higuchi et al., 2000). Therefore, these observations suggest that it is unlikely that the beneficial effects of Dimebon on cognition in patients with AD are mediated via the histaminergic pathway or the $H_1$ receptor.

Dimebon also binds to several $\alpha$-adrenoceptor subtypes ($\alpha_{1A}, \alpha_{1B}, \alpha_{1D}, \alpha_{2A}, \alpha_{2B}$, and $\alpha_{2C}$) that have been implicated in memory pathways (Arnsten and Cai, 1993; Arnsten et al., 1999). Whereas $\alpha_1$-adrenoceptor stimulation has been associated with impaired memory (Arnsten et al., 1999), $\alpha_2$-adrenoceptor stimulation has been associated with cognition enhancement (Arnsten and Cai, 1993). Further studies are needed to determine whether these $\alpha$-adrenoceptors mediate any of the cognition-enhancing effects of Dimebon.

Dimebon showed moderate affinity for the 5-hydroxytryptamine receptor subtype 6 (5-HT$_6$), and antagonists of this receptor have been associated with pro-cognitive effects in animals and humans (Hirst et al., 2006; Johnson et al., 2008). Schaffhauser et al. (2009) recently proposed that the cognitive-enhancing effects of Dimebon (dimebolin) may be mediated by the 5-HT$_6$ receptor. Although our studies cannot rule this out, it is unclear whether Dimebon levels in the brain that are achieved with low cognitive-enhancing doses (0.05 and 0.5 mg/kg) are sufficient to modulate the 5-HT$_6$ receptor. In addition, several studies have suggested that 5-HT$_6$ receptor antagonists mediate their cognition-enhancing effects by increasing extracellular ACh and glutamate levels in the brain (Riemer et al., 2003; Hirst et al., 2006; Upton et al., 2008). Because Dimebon did not increase extracellular brain ACh, the role of the 5-HT$_6$ receptor in mediating the cognition-enhancing effects of Dimebon in rats is unclear.

Previously reported in vitro studies suggest that Dimebon can enhance mitochondrial function and promote neuron activity (neurite outgrowth) in cultured hippocampal neurons (Hung, 2008; Bernales et al., 2009; Proter et al., 2009). Because mitochondria have been shown to be tightly coupled to spines and synapses (Li et al., 2004) and brain function (Gjedde, 2007; Sun and Cavalli, 2009), it is possible that the mitochondrial effects of Dimebon may be related to its cognitive effects. Mitochondrial dysfunction has been implicated in the pathogenesis of AD and HD (Bachurin et al., 2003; Chaturvedi and Beal, 2008; Mattson et al., 2008; Pavlov et al., 2009; Swerdlow and Khan, 2009). Dimebon has been shown to improve cognition in patients with AD (Doody et al., 2008) and HD (Kieburtz et al., 2010), suggesting the drug might affect important neuronal processes common to both diseases, such as mitochondria.

Although the precise mechanism for the pro-cognitive actions of Dimebon remains unknown, it appears to be distinct from the mechanisms of currently approved treatments for AD. This distinction provides a rationale for clinical trials that will study the combination of Dimebon with cholinesterase inhibitors, such as donepezil and galantamine, and with the NMDA receptor antagonist memantine. Further studies are also being conducted to determine the pharmacologic basis of the pro-cognitive effects of Dimebon in animals, as well as the cognitive, functional, and behavioral benefits observed in patients with AD.

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References


Gjedde A (2007) Coupling of brain function to metabolism: evaluation of energy requirements, in Brain Energetics Integration of Molecular and Cellular Processes (Gibson GE and Dincel GA eds), Springer, New York.

Goldman ME, Jacobson AE, Rice KC, and Paul SM (1985) Differentiation of
Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Nadarajah B (1992) The effect of pralidoxime chloride in the assay of acetylcholine-
Pavlov PF, Petersen CH, Glaser E, and Ankarcrona M (2009) Mitochondrial accu-
Paxinos G and Watson C (1998)
Alzheimer’s disease assessed by positron emission tomography.
J Physiol Pharmacol
Simons FE, Fraser TG, Reggin JD, and Simons KJ (1996) Comparison of the central
regeneration and degeneration.
Clin Exp Allergy
with a novel mitochondrial mechanism of action. Alzheimer Dementia
for the treatment of cognitive disorders including dementia. Curr Opin Drug
Nathraj B (1992) The effect of pralidoxime chloride in the assay of acetylcholinester-
ase using 5,5'-dithio-bis(2-nitrobenzoic acid) (Ellman’s reagent). J Anal Toxicol
Pavlov PF, Petersen CH, Glaser E, and Ankarcrona M (2009) Mitochondrial accumu-
Dis 6:A1536.
logical characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phe-
nylamine, a potent and selective 5-HT6 receptor antagonist. J Med Chem 46:1273–
1276.
hippocampal neurons following exposure to N-methyl-d-aspartate. J Physiol 448: 655–676.
26:1092–1097.
MCP900200.
22:663–672.
van Ruitenbeek P, Vermeeren A, and Riedel W (2008) Histamine H1-receptor block-
de in humans affects psychomotor performance but not memory. J Psychophar-
macol 22:663–672.
patients with mild to moderate Alzheimer’s disease: international randomised controlled trial. Galantamine International-1 Study Group.
Wu J, Li Q, and Bezprozvanny I (2008) Evaluation of Dimebon in cellular model of

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