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Lecozotan (SRA-333): A Selective Serotonin 1A Receptor Antagonist That Enhances the Stimulated Release of Glutamate and Acetylcholine in the Hippocampus and Possesses Cognitive-Enhancing Properties

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ABSTRACT

Recent data has suggested that the 5-hydroxytryptamine (5-HT)$_{1A}$ receptor is involved in cognitive processing. A novel 5-HT$_{1A}$ receptor antagonist, 4-cyano-N-{2R-[4-(2,3-dihydrobenzo[1,4]-dioxin-5-yl)-piperazin-1-yl]-propyl}-N-pyridin-2-yl-benzamide HCl (lecozotan), which has been characterized in multiple in vitro and in vivo pharmacological assays as a drug to treat cognitive dysfunction, is reported. In vitro binding and intrinsic activity determinations demonstrated that lecozotan is a potent and selective 5-HT$_{1A}$ receptor antagonist. Using in vivo microdialysis, lecozotan (0.3 mg/kg s.c.) antagonized the decrease in hippocampal extracellular 5-HT induced by a challenge dose (0.3 mg/kg s.c.) of 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) and had no effects alone at doses 10-fold higher. Lecozotan significantly potentiated the potassium chloride-stimulated release of glutamate and acetylcholine in the dentate gyrus of the hippocampus. Chronic administration of lecozotan did not induce 5-HT$_{1A}$ receptor toler-ance or desensitization in a behavioral model indicative of 5-HT$_{1A}$ receptor function. In drug discrimination studies, lecozotan (0.01–1 mg/kg i.m.) did not substitute for 8-OH-DPAT and produced a dose-related blockade of the 5-HT$_{1A}$ agonist discriminative stimulus cue. In aged rhesus monkeys, lecozotan produced a significant improvement in task performance efficiency at an optimal dose (1 mg/kg p.o.) Learning deficits induced by the glutamatergic antagonist MK-801 [(−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] (assessed by perceptually complex and visual spatial discrimination) and by specific cholinergic lesions of the hippocampus (assessed by visual spatial discrimination) were reversed by lecozotan (2 mg/kg i.m.) in marmosets. The heterosynaptic nature of the effects of lecozotan imbues this compound with a novel mechanism of action directed at the biochemical pathologies underlying cognitive loss in Alzheimer's disease.

The multiplicity of biological data associated with the 5-hydroxytryptamine (5-HT)$_{1A}$ receptor subtype, since its discovery by radioligand binding in 1981 (Pedigo et al., 1981) and subsequent cloning in 1988 (Fargin et al., 1988), implicates this receptor in numerous behavioral and physiological functions, including cognition, psychosis, feeding/satiety, temper-

ature regulation, anxiety, depression, sleep, pain perception, and sexual activity. The development of 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) as a selective agonist for the 5-HT$_{1A}$ receptor has been instrumental in defining the physiological role of the 5-HT$_{1A}$ receptor (Arvidsson et al., 1981) as well as its anatomic localization (Gozlan et al., 1983). However, it was not until the development of a "silent" 5-HT$_{1A}$ receptor antagonist that systematic pharmacological studies explored the consequences of blocking this receptor in the brain.

The first breakthrough in this area was reported in 1993 with the development of the arylpiperazine compound WAY-100135, which had moderate potency (Fletcher et al., 1993). The more potent and selective congener WAY-100635 was identified shortly thereafter (Forster et al., 1995). Since then, it has been demonstrated that WAY-100635 lacks intrinsic activity in multiple assay systems that are sensitive to the agonist effects of 5-HT$_{1A}$ compounds. Accordingly, WAY-100635 antagonizes the responses of 8-OH-DPAT at 5-HT$_{1A}$ somatodendritic autoreceptors to inhibit the firing rate of dorsal raphe neurons and antagonizes the ability of 8-OH-DPAT to decrease the accumulation of cAMP at postsynaptic 5-HT$_{1A}$ receptors in the hippocampus (Fletcher et al., 1996). Although WAY-100635 remains an important pharmacological tool in defining 5-HT$_{1A}$ receptor function, other 5-HT$_{1A}$ antagonists have been developed over the past decade, including WAY-405 (Schechter et al., 2000), which is another aryl-piperazine analog, and NAD-299 (Johansson et al., 1997) and LY-426965 [(2S)-1-cyclohexyl-4-[4-(2-methoxyphenyl)-1-piperazinyl]2-methyl-2-phenyl-1-butanone monohydrochloride] (Rasmussen et al., 2000), which are structural analogs of 8-OH-DPAT and pindolol, respectively.

One of the most intriguing areas of research surrounding 5-HT$_{1A}$ receptor antagonists is the potential of these compounds to enhance cognitive ability. AD has been described as a neurodegenerative disorder characterized by multiple deficits in neurotransmitter function. Although many studies have focused upon the cholinergic hypothesis, it has become apparent that not all patients can be characterized by deficits in this system alone, as shown by the moderate efficacy produced by acetylcholinesterase inhibitors (Morris et al., 1986; Bliss and Collingridge, 1993). In fact, recent research suggests that glutamatergic deficits may occur before those observed in the cholinergic system (Bliss and Collingridge, 1993). Furthermore, and in contrast to glutamatergic and cholinergic neurotransmission, the serotonergic system may be hyperactive in the disease as a result of the enhanced turnover of serotonin (Kowall and Beal, 1991), which ultimately would reduce the firing of the cortical pyramidal-associated pathways through stimulation of 5-HT$_{1A}$ autoreceptors. It was this finding of enhanced serotonergic turnover that lead Bowen et al. (1994) to the hypothesis that 5-HT$_{1A}$ receptor antagonists may be effective in treating the cognitive loss associated with AD.

A compelling rationale has been proposed for treating the dementia associated with AD based on data that antagonists of 5-HT$_{1A}$ receptors have a facilitatory effect on glutamatergic transmission (Bliss and Collingridge, 1993; Bowen et al., 1994). N-Methyl-D-aspartate (NMDA)-induced glutamate release from pyramidal neurons is potentiated by a 5-HT$_{1A}$ receptor antagonist (Dijk et al., 1995). The 5-HT$_{1A}$ antagonist WAY-100635 can alleviate cognitive deficits induced by both glutamatergic dysfunction (dizocilpine treatment-induced cognitive deficits) and cholinergic dysfunction (fornix transaction surgery) in primates (Harder et al., 1996; Harder and Ridley, 2000). The blockade of 5-HT$_{1A}$ receptors also enhances glutamate release from rat hippocampal slices (Van den Hooff and Galvan, 1991). Other data suggest that 5-HT$_{1A}$ receptor antagonists can inhibit the tonic hyperpolarizing action of serotonin on pyramidal neurons in both the cortex and hippocampus (Araneda and Andrade, 1991; Van den Hooff and Galvan, 1992), which ultimately would result in an enhancement of glutamatergic neurotransmission and signaling. Thus, it is possible that 5-HT$_{1A}$ receptor antagonists may improve cognition by removing the inhibitory effects of endogenous serotonin on pyramidal neurons and thus enhance glutamatergic activation and ensuing signal transduction.

The purpose of this study was to investigate the pharmacological characteristics of SRA-333 (lecozotan), a novel, selective, and potent antagonist of 5-HT$_{1A}$ receptors. Lecozotan specifically antagonizes functional responses associated with stimulating the 5-HT$_{1A}$ receptor both in vitro and in vivo. The ability of lecozotan to potentiate the release of acetylcholine and glutamate in vivo as well as its positive role in relevant biological models for cognition supports the utility of this compound as a novel strategy to treat the cognitive deficits associated with AD.

Materials and Methods

Radioligand Binding

Membrane Preparations. *5-HT$_{1A}$ receptor.* The polymerase chain reaction cloning of the human 5-HT$_{1A}$ receptor subtype from a human genomic library has been described previously (Chanda et al., 1993). A stable Chinese hamster ovary cell line (CHO-K1) expressing the human 5-HT$_{1A}$ receptor subtype (h5-HT$_{1A}$ CHO cells) was used throughout this study. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, nonessential amino acids, and penicillin/streptomycin. Cells were grown to 95 to 100% confluence as a monolayer before membranes were harvested for binding studies. Cells were gently scraped from the culture plates, transferred to centrifuge tubes, and washed twice by centrifugation (2000 rpm for 10 min at 4°C) in buffer (50 mM Tris, pH 7.5). The resulting pellets were aliquoted and placed at −80°C. On the day of assay, the cells were thawed on ice and resuspended in buffer.

Dopamine receptors. The binding profile of lecozotan was assessed in stable CHO-K1 cells containing dopamine D$_2$ (DA)$_2$, dopamine D$_3$ (DA$_3$), and dopamine D$_4$ (DA$_4$) receptor subtypes. The CHO-K1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, nonessential amino acids, and penicillin/streptomycin. The cells were grown to 95% confluence as a monolayer and were harvested by scraping. The harvested cells were centrifuged at low speed (1000g) for 10 min to remove the culture media. The harvested cells were suspended in half-volume of fresh physiological phosphate-buffered saline solution and recentrifuged at the same speed. This operation was repeated once more. The collected cells were then homogenized in 10 volumes of 50 mM Tris-HCl, pH 7.4, and 0.5 mM EDTA. The homogenate was centrifuged at 40,000g for 30 min, and the precipitate was collected. The pellet was resuspended in 10 volumes of Tris-HCl buffer and recentrifuged at the same speed. The final pellet was suspended in a small volume of Tris-HCl buffer, and the tissue protein content was determined in aliquots of 10- to 25-μl volumes. Bovine serum albumin was used as the standard in the protein determination by the method of Lowry et al. (1951). The volume of the suspended cell membranes was adjusted to give a tissue protein concentration of 1.0 mg/ml

suspension. The prepared membrane suspension (10 times concentrated) was aliquoted in 1.0-ml volumes and stored at −70°C until used in subsequent binding experiments.

α_1 Adrenergic receptor. Cortical tissue from male Sprague-Dawley rats was homogenized on ice in 50 mM Tris-HCl, pH 7.4, buffer with a Polytron homogenizer. The homogenate was then centrifuged at 25,000*g* for 10 min, and the supernatant was discarded. The pellet was resuspended in fresh buffer, centrifuged, and then resuspended. After a third wash of the membranes, the pellet was resuspended in buffer, aliquoted into separate vials, and stored at −70°C until subsequent assay.

Radioligand Binding Protocols. *$5\text{-}HT_{1A}$ receptor binding.* [^{3}H]WAY-100635 and [^{3}H]8-OH-DPAT were used to label $5\text{-}HT_{1A}$ receptors. Experiments were conducted in 96-well microtiter plates in a total volume of 250 μl of buffer (50 mM Tris-HCl, pH 7.4). Nonspecific binding was defined with 10 μM methiothepin for [^{3}H]WAY-100635 binding. Competition studies were conducted using 1 nM ligand. For [^{3}H]8-OH-DPAT binding, nonspecific binding was defined with 10 μM serotonin. Competition studies were conducted using 1.50 nM of the ligand. The binding assays were initiated by the addition of 50 μl of the harvested stabile transfected $5\text{-}HT_{1A}$ cells (0.05 mg/sample) and were incubated at 25°C for 30 min. The reaction was terminated by vacuum filtration through presoaked (0.5% polyethyleneimine) Whatman GF/B filter paper (Brandel Inc., Gaithersburg, MD) using a Brandel 96-cell harvester. Filters were washed with ice-cold buffer (50 mM Tris-HCl, pH 7.4) and transferred to scintillation vials, to which 5 ml of Opti-Fluor (PerkinElmer Life and Analytical Sciences, Boston, MA) was added. Radioactivity was measured by liquid scintillation counting using a Beckman LS 6000TA liquid scintillation counter (Beckman Coulter Inc., Fullerton, CA). Protein concentrations were determined by the method of Bradford using bovine serum albumin as the standard. Measurements were taken at 595 nm with a PerkinElmer Lambda 3B spectrophotometer (PerkinElmer Life and Analytical Sciences). Binding data were analyzed by ReceptorFit (Lundon Software, Cleveland Heights, OH), a computer-assisted nonlinear regression analysis program.

Dopamine receptor binding. The radioligand [^{3}H]spiperone was used at 0.5 nM to label DA_2, DA_3, and DA_4 receptors. Nonspecific binding was determined in the presence of 10 μM *d*-butaclamol; 7-hydroxy-DPAT; or clozapine for DA_2, DA_3, and DA_4, respectively. Binding experiments were performed in a 96-well microtiter plate format, in a total volume of 200 μl. Competition experiments were performed using seven to nine concentrations of lecozotan, added in 20-μl volume. To each well was added 80 μl of incubation buffer made in 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM $MgCl_2$ and 0.5 mM EDTA, and 20 μl of radioisotope. The reaction was initiated by the final addition of 100 μl of tissue suspension. The reaction proceeded in the dark for 120 min at room temperature, at which time the bound ligand-receptor complex was filtered off on a 96-well unifilter with a Filtermate 196 harvester (PerkinElmer Life and Analytical Sciences). The radioactivity of the bound complex caught on the filter disk was measured after drying and addition of 40 μl of Microscint-20 scintillant to each well in a TopCount (PerkinElmer Life and Analytical Sciences) equipped with six photomultiplier detectors. The unifilter plate was heat sealed and counted in a TopCount with efficiencies of 31.0 and 60% for tritium and [^{125}I], respectively.

α_1 Adrenergic receptor binding. Membranes were thawed, placed in buffer, and incubated with [^{3}H]prazosin (0.2 nM) for 30 min at 25°C. All tubes contained either vehicle, test compound (one to eight concentrations), or a saturating concentration of phentolamine (10 μM) to define specific binding. All reactions were terminated by the addition of ice-cold Tris buffer followed by rapid filtration using a TomTech filtration device (TomTech, Hamden, CT) to separate bound from free [^{3}H]prazosin. Bound radioactivity was quantitated using a Wallac 1205 Beta Plate counter (PerkinElmer Life and Analytical Sciences).

Nova Screen binding profile. A Nova Screen binding profile was determined for lecozotan at 61 neurotransmitter receptor, reuptake, ion channel, and enzyme sites. [^{3}H]8-OH-DPAT and [^{3}H]WAY-100635 were used as the $5\text{-}HT_{1A}$ agonist radioligand and the $5\text{-}HT_{1A}$ antagonist radioligand, respectively. Displacement of specific radioligand binding at these sites was determined at three concentrations of lecozotan (1, 100, and 10,000 nM).

Determination of Intrinsic Activity Using a cAMP Accumulation Assay

Assays were performed by incubating the cells with Dulbecco's modified Eagle's medium containing 25 mM HEPES, 5 mM theophylline, and 10 μM pargyline for a period of 20 min at 37°C. Functional activity was assessed by treating the cells with forskolin (1 μM final concentration) followed immediately by a test compound (six concentrations) for an additional 10 min at 37°C. In separate experiments, six concentrations of antagonist were preincubated for 20 min before the addition of 10 nM 8-OH-DPAT (Sigma/RBI, Natick, MA) and forskolin. The reaction was terminated by removal of the media and addition of 0.5 ml of ice-cold assay buffer. Plates were stored at −20°C before the assessment of cAMP formation by a cAMP SPA assay (Amersham Biosciences Inc., Piscataway, NJ).

Assessment of Intrinsic Activity Using [^{35}S]GTPγS Binding

The guanosine 5′-*O*-(3-thio)triphosphate ([^{35}S]GTPγS) binding assay was similar to that used by Lazareno and Birdsall (1993). Briefly, $5\text{-}HT_{1A}$-cloned receptor membrane fragments (as used for $5\text{-}HT_{1A}$ receptor binding assays) were stored at −70°C until needed. When needed, membranes were rapidly thawed, centrifuged at 40,000*g* for 10 min, and resuspended at 4°C for 10 min in assay buffer [25 mM HEPES, 3 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 10 μM GDP, and 500 mM dithiothreitol (Cleland's Reagent), pH 8.0]. These membranes were then incubated for 30 min at 30°C with [^{35}S]GTPγS (1 nM) in the presence of vehicle, test compound (one to eight concentrations), or excess 8-OH-DPAT to define maximum agonist response. All reactions were terminated by the addition of ice-cold Tris buffer followed by rapid filtration using a TomTech filtration device to separate bound from free [^{35}S]GTPγS. Agonists produced an increase in the amount of [^{35}S]GTPγS bound, whereas antagonists produced no increase in binding. Bound radioactivity was counted and analyzed as described above.

In Vivo Microdialysis: 5-HT and Glutamate

Subjects and Surgery. Male Sprague-Dawley rats (280–350 g; Charles River Laboratories, Inc., Wilmington, MA) were used in all experiments. Animals were housed as a group with food and water available ad libitum and maintained on a 12-h light/dark cycle (lights on at 6:00 AM) with all work performed during the light phase. After surgery, the animals were housed separately in Plexiglas cages (45 × 45 × 30 cm) with free access to food and water.

Microdialysis Procedures. After induction of anesthesia with gaseous administration of halothane (2%) (Fluothane; Zeneca, Cheshire, UK), the animals were secured in a stereotaxic frame with ear and incisor bars. Anesthesia was maintained by continuous administration of halothane (1 to 2%). A microdialysis probe guide cannula (CMA/Microdialysis, Stockholm, Sweden) was implanted into either the ventral hippocampus or the dentate gyrus. Coordinates for the both brain regions were taken from Paxinos et al. (1985): RC, −4.8; L, −5.0; and V, −4.4 and RC, −3.8; L, −1.4, and V, −3.6, respectively; reference points for microdialysis cannula implantation were taken from bregma (RC and L) and the dura (V). A cannula was also implanted s.c. at this time between the animal's shoulders. Both cannulae were secured to the skull using dental acrylic (Plastics One, Roanoke, VA). The wound was sutured and the animals left to recover for 24 h in their home cages with free access to food and water.

A pre-equilibrated microdialysis probe (optical density 0.5 mm,

membrane length 4 or 1 mm; CMA/Microdialysis), perfused overnight in artificial cerebrospinal fluid, was inserted, via the guide cannula, into either the ventral hippocampus or the dentate gyrus, respectively, of the unrestrained rat 24 h after surgery. The probe was perfused with aCSF (125 mM NaCl, 3.0 mM KCl, 0.75 mM MgSO$_4$, and 1.2 mM CaCl$_2$, pH 7.4) at a flow rate of 1 μl/min. A 3-h stabilization period was allowed after probe implantation, after which time microdialysis sampling was carried out by a modification of the method of Dawson and Routledge (1995). For 5-HT analysis, 20-min samples were taken throughout the experimental period, and four preinjection samples were taken before drug administration. Alternatively, for glutamate determinations, a 5-min sampling regimen was used throughout the experimental period, and six presamples were taken before drug or vehicle injection to achieve a steady baseline. All subsequent samples were expressed as a percentage of these baseline concentrations. Lecozotan or vehicle was administered via the cannula (s.c.). Stimulation of neurotransmitter release was brought about by infusion of aCSF containing 100 mM KCl (28 mM NaCl, 100 mM KCl, 0.75 mM MgSO$_4$, and 1.2 mM CaCl$_2$, pH 7.4) through the microdialysis probe.

Analysis of Microdialysates. *5-HT analysis.* 5-HT was separated by reverse-phase high-performance liquid chromatography (HPLC) (C18 ODS2 column, 100 × 3.0 mm; Metachem, Torrance, CA) and detected using an ANTEC electrochemical detector (ANTEC, Leiden, The Netherlands) set at a potential of 0.65 V versus a Ag/AgCl reference electrode. Mobile phase was delivered by a Jasco PU980 HPLC pump (Jasco Ltd., Essex, UK) at 0.6 ml/min and contained 0.15 M NaH$_2$PO$_4$ buffer at pH 4.3, 0.25 mM EDTA, 1.5 mM 1-octane sodium sulfonic acid, and 5% isopropanol.

Glutamate analysis. Measurement of glutamate was performed using a Crystal 310 capillary electrophoresis system (Thermo Bio-Analysis, Sante Fe, NM) with a Zeta laser-induced fluorescence detector (ZETA Technology, Toulouse, France) coupled with a Omnichrome helium-cadmium laser (emission wavelength 442 nm; Melles Griot, Carlsbad, CA). All samples were prederivatized with naphthalene 2,3-dicarboxaldehyde by a modification of the method of Hernandez et al. (1993). Microdialysate or standard samples (3 μl) were mixed with 50 mM boric acid buffer, pH 9.5, containing 20 mM sodium cyanide (5 μl) and 30 mM naphthalene 2,3-dicarboxaldehyde in methanol (1 μl). Samples were allowed to react for 3 min at room temperature before injection. Separations were performed according to Dawson et al. (1997) in fused silica capillaries (75 μm i.d., 375 μm o.d., 47 cm; Polymicro Technologies, Phoenix, AZ) with an applied voltage of 0.6 kV/cm. Samples (5 nl) were applied to the capillary via a high-pressure injection system. Separations used 30 mM boric acid, pH 9.5 (pH was adjusted using 1 M NaOH). The capillary was rinsed with 0.1 M NaOH (1.5 min) and running buffer (1.5 min) between analyses. All data were acquired using the Atlas software package (Thermo Labsystems, Gulph Mills, PA) for the PC.

The femtomoles per micromolar perfusate values of 5-HT/glutamate for the first four baseline samples were averaged, and this value was denoted as 100%. Results were analyzed by analysis of variance (ANOVA) with repeated measures followed by pairwise comparisons using Bonferroni adjustment for multiple comparisons using the Statview software application (Abacus Concepts, Berkeley, CA) for the PC.

In Vivo Microdialysis: Acetylcholine

Subjects and Surgery. Male Sprague-Dawley rats weighing between 350 to 400 g were maintained in individual cages. Animals were anesthetized with a combination of ketamine (50 mg/kg i.p.) and xylazine (10 mg/kg i.p.), and a single guide shaft aimed at the hippocampus (equal right and left side implants; region CA1) was implanted (coordinates RC, −5.6; L, 5.0; and V, 3.0 mm). Rats were allowed to recover at least a week before experimentation.

Microdialysis Procedure. A concentric microdialysis probe with a 4-mm cellulose tip was inserted in the hippocampus of each rat 14 h before the experiment. The probe was perfused with a modified Ringer's solution (142 mM NaCl, 3.9 mM KCl, 1.2 mM CaCl$_2$, 1.0 mM MgCl$_2$, and 10 mM bicarbonate, pH 7.4) at a flow rate of 0.5 μl/min overnight and 1 μl/min starting 1 h before the experiment. To enhance acetylcholine recovery, it was necessary to add a very small concentration of neostigmine (0.3 μM) to the Ringer's solution. Samples were collected every 20 min before and after injection of lecozotan.

Analysis of microdialysates. Acetylcholine was measured by reverse-phase high-performance liquid chromatography with electrochemical detection using an ESA model 580 pump and mobile phase of 200 mM potassium phosphate, pH 8.0, at a flow rate of 0.6 ml/min (Rada et al., 2001). Microdialysates were injected into a 20-μl loop leading to a 10-cm C18 analytical column to separate acetylcholine, which was converted to betaine and hydrogen peroxide by an immobilized enzyme reactor. Detection was accomplished with an amperometric detector (model 400; PerkinElmer Life and Analytical Sciences) that oxidized the hydrogen peroxide on a platinum electrode (BAS Bioanalytical Systems, West Lafayette, IN) set at 0.5 V with respect to a Ag/AgCl reference electrode.

In Vivo Electrophysiology

Male Sprague-Dawley rats (240–265 g; Laboratories, Inc.) were housed in groups on a 12-h light/dark cycle with ad libitum access to food and water. On the day of the experiments, rats were initially anesthetized with halothane in an induction chamber. Anesthesia was subsequently maintained by continuously administering halothane (in oxygen at 1 to 3%) through a nose cone using a Fluotec halothane vaporizer. A heating pad was placed beneath the animal to maintain the body temperature at 37.5°C.

Rats were placed in a stereotaxic frame and the surface of the skull was exposed. Glass micropipettes (filled with pontamine sky blue) with an impedance of 3 to 6 megaohms were lowered through a craniotomy to a depth of 0.5 mm above the dorsal raphe nucleus (on the midline and −0.6 mm from the interaural zero). A hydraulic drive was used to further advance the recording electrode. Neuronal action potentials were passed through a high-input impedance amplifier and were monitored on an oscilloscope. Action potentials were discriminated from background noise and used to trigger an output, which was counted and recorded by an online computer system (DataWave Technology, Longmont, CO).

Dorsal raphe neurons were identified by characteristic wave form activity and then spontaneous activity was monitored for approximately 10 min to establish a baseline rate of neuronal firing. A predetermined dose of the test compound was then administered subcutaneously and firing rate was monitored for an additional 3 min to observe any effect of the test compound. 8-OH-DPAT was then administered s.c. in cumulative doses at 3-min intervals. Only one neuron was studied in each rat to avoid residual effects. At the termination of the experiment, the pontamine sky blue was deposited for 20 min by a 10-μA anodal current for histological confirmation of the recording site. Each animal was then perfused intracardially with Mirsky's formula, after which the brain was removed, sectioned at 64 μm, and counterstained with neutral red. Data from recording sites not found within the histological boundaries of the dorsal raphe were discarded.

All drugs were put into solution with 0.9% saline. The single pretreatment dose of lecozotan was in a volume concentration of 300 μg/kg/0.1 ml. 8-OH-DPAT was injected in cumulative doses starting at 25 μg/kg in a volume of 0.05 ml. All doses were expressed on the basis of active moiety. Firing rates for the baseline period and that after administration of lecozotan were compared using a paired t test ($p < 0.05$).

Fixed Ratio (FR) Operant Studies in Rats

Male Sprague-Dawley rats weighing 300 to 350 g were housed individually and maintained at 85% of their free-feeding body

weights by food presented during the session and by postsession feeding. Water was freely available in the animal's home cage.

Experimental sessions were conducted in a standard operant conditioning chamber placed inside a ventilated sound-attenuating shell that was equipped with white noise to mask extraneous sounds (MED Associates, Georgia, VT). A response lever and a food trough were on the front panel of the chamber. Bioserv (Frenchtown, NJ) 45-mg precision dustless pellets could be delivered to food troughs to serve as reinforcers. The operant chambers were controlled and monitored by computers with software from MED Associates.

Rats were trained to respond on the right lever under an FR30 schedule of food presentation. Experimental sessions consisted of three 10-min components, each preceded by a 10-min time-out period during which drugs were administered. During the time-out period, the chamber was dark, and there were no programmed consequences. During the response component, the house light was illuminated, and lever pressing was associated with an audible feedback click. 8-OH-DPAT (0.03–0.3 mg/kg) was administered cumulatively s.c. at the start of the time-out periods. Lecozotan was administered as a 30-min pretreatment before a cumulative 8-OH-DPAT dose-effect curve. The ED_{50} was defined as the dose of 8-OH-DPAT that produced a 50% reduction in the response rate in the presence or absence of lecozotan. Data from multiple administrations were overlapped and combined. Rates of responding were calculated separately in each of the three components of the session by dividing the total number of responses by the total time the component was in effect for each animal.

Fixed Ratio Operant Studies in Squirrel Monkeys

Four adult male squirrel monkeys, *Saimiri sciureus*, were housed in individual cages except during experimental sessions. Each monkey had unlimited access to water and received a nutritionally balanced diet of Purina Monkey Chow, fresh fruits, vegetables, and vitamin supplements.

Experimental sessions were conducted in ventilated sound-attenuating chambers (MED Associates) that were provided with white noise to mask extraneous sounds. Monkeys sat in a Plexiglas chair similar to one used by Kelleher et al. (1972) and faced a panel on which a response lever and colored stimulus lamps were mounted. Each press of the lever with a minimal downward force of 0.25 N was recorded as a response. A shaved portion of the tail of the monkey was secured in a stock beneath brass electrodes. Electrode paste insured a low-resistance contact between the electrodes and the tail. A brief, low-intensity electric shock (200 ms; 3–5 mA) could be delivered through the electrodes to the tail.

Responding was maintained by termination of a visual stimulus associated with an electric shock (Kelleher et al., 1972). In the presence of a red light, the completion of a 10-response fixed ratio unit (FR10) turned off the red light and initiated a 30-s time-out period. If the FR was not completed within a 10-s period, an electric shock was delivered every 10 s; a maximum of 10 shocks was delivered. Daily sessions consisted of five components. Each component consisted of a 10-min time-out period during which drugs could be administered, followed by a 5-min response period during which the FR10 schedule was in effect. Responding during the time-out period had no programmed consequences.

Incremental doses of 8-OH-DPAT were administered cumulatively i.m. at the start of the 10-min time-out period that preceded each of the five sequential components. Lecozotan was administered 30 min i.m. or 60 min p.o. before the start of the first response component.

Rates of responding were calculated separately in each of the five components of the session by dividing the total number of responses by the total time the component was in effect for each animal. ED_{50} values were determined for the effects of 8-OH-DPAT alone and after pretreatment with lecozotan.

Discriminative Stimulus Effects in Pigeons

Six male white Carneaux pigeons, approximately 1-year old, were obtained from the Palmetto Pigeon Plant (Sunter, SC). All pigeons were experimentally naive and were maintained individually in cages that were provided with continuously available water and grit. Lighting in the temperature- and-humidity-controlled vivarium was on a 12-h light/dark cycle. All pigeons were reduced to approximately 85% of their free-feeding body weights before to key peck training and were maintained at this weight for the duration of the study.

Experimental sessions were conducted in a standard operant conditioning chamber placed inside a ventilated sound-attenuating shell that was equipped with white noise to mask extraneous sounds (MED Associates). The front panel of the chamber contained three response keys. The keys could be transilluminated with different colors. The left and right keys were lit white and used in the present study. Pecks that exceeded approximately 0.15 N on the key operated a feedback relay behind the front wall and were counted as a response. Below the center key was a rectangular opening (4.5 × 10 cm) that provided access to a solenoid-driven food magazine containing mixed grain. During food delivery, the magazine was illuminated.

Pigeons were trained to discriminate 8-OH-DPAT (0.1 mg/kg i.m.) from saline using a two-key grain-reinforced drug discrimination procedure. Pigeons were initially trained to key peck on a FR schedule of food presentation (only one key illuminated at a time) with a gradual increase in the FR (final FR = 30) over the training sessions. Pigeons were then trained to respond on one key after a saline (s) injection (i.m.) and on the other key after an 8-OH-DPAT (d) injection (i.m.) using a double-alternation daily injection schedule (s, d, d, s, s, d). When the injection schedule started, both keys were lit, and the FR was dropped to 5, with a gradual increase to FR30. At the final schedule, completion of 30 consecutive responses on the injection-appropriate key resulted in 3-s access to mixed grain. Responding on the incorrect key reset the FR for the injection-appropriate key. Training and test sessions were 30 min in duration. Criteria for establishing 8-OH-DPAT as a discriminative stimulus were that the first ratio (30 consecutive responses) was completed on the injection-appropriate key and that there was >90% injection-appropriate response for the total session for five consecutive sessions.

Antagonism studies were conducted once or twice a week in individual pigeons. For antagonism studies, lecozotan (0.01–1.0 mg/kg i.m.) was administered as a pretreatment (40 min before test session) followed by an injection (i.m.) of the training dose of 8-OH-DPAT (0.1 mg/kg 30 min before test session). On test days, completion of 30 consecutive responses on either key resulted in access to mixed grain. Test sessions were conducted only if a pigeon met the criteria (first ratio correct and >90% responding on the injection-appropriate key) on the previous day and on four of the five previous training days. Training sessions did not take place on days after test sessions to allow for a washout period for test compounds.

Rates of responding were calculated by dividing the total number of responses by the total time of the session. Baseline drug key rates were calculated by averaging rates of drug injection days preceding a test day. Baseline saline key rates were calculated by averaging rates of saline injection days preceding a test day. The percentage of drug lever responding was calculated by dividing the number of responses on the drug-appropriate key by the total number of responses in the session multiplied by 100. Compounds were considered to fully attenuate the effects of the training dose of 8-OH-DPAT in an individual animal if drug key responding was reduced to <20%. Compounds were considered to partially attenuate the effects of the training dose of 8-OH-DPAT in an individual animal if the drug key responding was reduced to 20 to 60%. If an animal did not receive a reinforcer during a test session, the percentage of drug key responding was not analyzed. However, the rate of responding was included in the analysis.

Antagonism of 5-Methoxy-*N,N*-dimethyltryptamine (5-MeODMT)-Induced Syndrome

Male Sprague-Dawley rats (200–350 g) were housed in groups (four/cage) under a 12-h light/dark cycle with food and water available ad libitum. Serotonin syndrome tests were conducted according to a modification of the procedure by Smith and Peroutka (1986). Scoring the serotonin syndrome consisted of rating each of the following behaviors: 1) forepaw treading, 2) headweaving, 3) tremor, 4) hindlimb abduction, 5) flattened body posture, and 6) Straub tail on a four-point (0 to 3) ranked intensity scale (maximum score 18). To evaluate acute effects, lecozotan or vehicle was administered (p.o.) to rats (*n* = 8/dose) placed individually in observation cages (9.5 in width × 7.5 in height × 17.5 in length) lined with corncob bedding. Agonist activity was evaluated by scoring subjects for the presence of the serotonin syndrome during the final 15 min of the 1-h drug pretreatment interval. Antagonist activity was then determined by scoring for the presence of the serotonin syndrome for 15 min after challenging (i.p.) the subjects with 5-MeODMT (5.6 mg/kg), a non-selective 5-HT agonist. This agonist was used because it produced a more robust syndrome that could be completely blocked by full antagonists of the 5-HT$_{1A}$ receptor. In a separate experiment, lecozotan (10 mg/kg) or vehicle (1 ml/kg) was administered (p.o.) once daily for seven consecutive days. Twenty-four hours after the last dose, the potency of 5-MeO-DMT (0.3–5.6 mg/kg; *n* = 8 per dose) given i.p. was determined in a dose-response study in rats receiving either chronic vehicle or chronic lecozotan. In addition, the potency of lecozotan (1–17 mg/kg; *n* = 8 per group) given p.o. to antagonize a challenge dose of 5-MeO-DMT (5.6 mg/kg i.p.) was evaluated in a separate group of rats receiving p.o. chronic vehicle or lecozotan (10 mg/kg).

Statistically significant effects were determined by ANOVA followed by Dunnett's test ($p < 0.05$). ED$_{50}$ values were determined using nonlinear regression analysis with inverse prediction.

Aged Rhesus Monkey Task Performance

Male (two) and female (three) rhesus monkeys aged 21 to 29 years were well trained (>100 individual sessions) in the delayed matching-to-sample (DMTS) task. The animals were maintained on tap water (unlimited) and standard laboratory monkey chow supplemented with fruits and vegetables. They were maintained on a feeding schedule such that approximately 15% of their normal daily (except weekends) food intake was derived from 300-mg reinforcement food pellets (commercial composition of standard monkey chow and banana flakes) obtained during experimental sessions. The remainder was made available after each test session. On weekends, the animals were fed twice per day. The monkeys were maintained on a 12-h light/dark cycle and were tested each weekday between 9:00 AM and 2:00 PM. Room temperature and humidity was maintained at 72 ± 1°C and 52 ± 2%, respectively. The aged female animals in the study were perimenopausal, i.e., still cycling, but infrequently. Testing was performed between menstrual cycles.

Animals were tested simultaneously in their home cages using a computer-automated training and testing system. Daily sessions consisted of 96 trials. Each test unit included four press-key/pellet dispenser units (MED Associates) mounted in lightweight aluminum chassis that could be attached to the home cage. A trial began by the illumination of the sample press key located in the upper center of the panel. Monkeys were trained to press the illuminated sample (red, green, or yellow) area to initiate a trial. This action also extinguished the sample during a computer-specified delay interval. After the delay interval, the choice press keys (two), but not the sample, were illuminated. One of the two choices matched the sample, whereas the other (incorrect) choice was presented as one of the two remaining colors. Correct trials (matches) were rewarded by the delivery of a reinforcement food pellet (intertrial interval 5 s). The various combinations of stimulus color were arranged so that each occurred an equal number of times as a sample, each color occurred an equal number of times as choices, and each color occurred an

equal number of times in combination with each other color. Likewise, when two colors occurred in combination, each color was counterbalanced between left and right sides of the screen in a nonpredictable pattern. Finally, all stimulus-counterbalancing procedures were matched to length of delay interval. For each subject, baseline accuracy was normalized by adjusting the length of delay intervals to provide: zero delay (85–100% correct); short delay (75–84% correct); medium delay (65–74% correct), and long delay (55–64% correct). Baseline data were obtained after the administration of a drug vehicle (sterile saline).

Data for percentage of correct were subdivided according to delay interval for each 24-trial delay component of the session. All statistical analyses were performed on raw data (percentage of trials correct). Data were analyzed by a multifactorial ANOVA with repeated measures (SAS; JMP statistical software package). An orthogonal multicomparison *t* test was used to compare individual means. Differences between means from experimental groups were considered significant at the $p < 0.05$ level (two-sided test).

Cognitive-Enhancing Profile of Lecozotan in Marmosets

Animals. Common marmosets (*Callithrix jacchus*), bred and housed in the primate colony at the University of Bradford School of Pharmacy, were used for these studies. All marmosets were housed in mixed sex pairs, in which the male animals were vasectomized at least 3 months before any behavioral studies. Marmosets were fed and watered ad libitum throughout the studies. Their diet consisted of a mixture of commercially available primate "rings" and primate "forage mix" (Harlan UK Limited, Bicester, Oxon, UK), plus varied fruits, multiseed bread, and occasional sweet treats in the form of gum feeders.

Treatments. All drugs were administered (i.m.) with saline as the vehicle. Injection volumes were kept at a maximum of 0.1 ml per marmoset per drug per day. MK-801 (dizocilpine maleate) was obtained from Sigma-Aldrich (St. Louis, MO). Both drugs were diluted in 0.9% sterile saline. Dizocilpine was administered at a dose of 15 μg/kg. Lecozotan was administered at doses of 1 and 2 mg/kg. Dizocilpine was administered as a 30-min pretreatment on each day when animals were learning tasks under either dizocilpine treatment alone or dizocilpine + lecozotan treatment. Lecozotan was administered as a 20-min pretreatment on days when animals were learning tasks under either lecozotan treatment alone or under dizocilpine + lecozotan treatment.

All animals received all treatments in a counter-balanced order. Dosing was semichronic; e.g., when an animal took 2 weeks to learn a task, that animal would receive 2 weeks of daily injections during the period when the task was being acquired. If an animal took a month to learn a task, then drugs were administered throughout the month of testing on a daily basis.

Lesion Surgery. We have previously demonstrated that a significant and highly specific lesion of the marmoset vertical diagonal band (VDB), the source of the majority of cholinergic input to the hippocampus, is possible using a p75-saporin conjugate (Harder et al., 1998). An antibody to the p75 human low-affinity nerve growth factor receptor, thought to be found mainly on cholinergic cell bodies, is conjugated to the neurotoxin saporin. This preparation is commercially available through Advanced Targeting Systems as ME 20.4-SAP. The preparation was diluted to 2 μg/μl in sterile 0.9% saline.

Marmosets were sedated with 0.05 ml of ketamine solution (Vetalar; Aveco Co. Inc., Fort Dodge, IA), before being weighed and anesthetized with Saffan. Marmosets were placed in a pouch of bubble wrap on a warmed operating table with their heads placed in a stereotaxic frame. A small (approximately 2.5 cm) incision from the center of the forehead running posteriorly to the back of the head was made, and the skin retracted on either side. The stereotaxic frame was centered to bregma, and burr holes were drilled using a small dental drill to allow placement of 1.5 μl of immunotoxin in each hemisphere at the following coordinates: AP, +1.15 cm; L, ±0.08 cm; V, +0.83 cm and AP, +1.20 cm; L, ±0.05 cm; V, +0.98 cm. After

suturing and recovery in a heated secure box, marmosets were returned to the home cage for behavioral testing, beginning 2 weeks postoperatively.

Lesion Verification. Upon completion of the study the lesions administered to the subjects were verified to ensure bilateral lesions had been achieved in the marmosets. Lesion verification was achieved exactly as described by the method of Ridley et al. (1999). Briefly, after behavioral testing was completed the lesion procedure was validated using an antibody stain for the p75 nerve growth factor expressed on cholinergic neurons. Sections were examined under a light microscope at $25\times$ magnification. All animals that were tested in the behavioral procedure were validated and had a complete bilateral loss of cholinergic staining in the hippocampi, which correlated with the cognitive deficits observed.

Behavioral Testing. Previous studies in many laboratories have made extensive use of the Wisconsin General Test Apparatus (WGTA) adapted for use with marmosets. Two WGTA task types used in these studies are described below. Their neural substrates have been well delineated (Harder et al., 1998; Ridley et al., 1999).

Cortical task: the perceptually complex visual discrimination task. Previously, we have shown that acquisition of perceptually simple visual discrimination tasks is not robustly affected by dizocilpine, but if the simple visual discrimination task is made more perceptually difficult, a robust deficit is seen (Harder et al., 1998). Hence, in this study marmosets were only tested on perceptually complex version tasks. Objects were painted matte black using car spray paint, such that only the shape could be used to distinguish between different objects (color and textural differences being eliminated through the painting).

Two different objects were presented to the marmoset in the WGTA: one situated over the right food well, and the other over the left food well. For every task, two different objects were used, and in each case, only one of the objects was associated with a reward. Thus, for example, object A might be the cap of a pen, painted black, and object B might be the small plastic toy from a cereal box, also painted black. On any single trial, the marmoset would see the two objects, A and B, positioned according to a pseudorandom schedule over the right and left food wells. On trial 1, A (the pen cap) might be on the right, and B (the toy) on the left. On trial 2, B might be on the right and A on the left.

Marmosets chose an object by moving the object to reveal the reward underneath, if the correct object was chosen. In the case of visual discrimination tasks, the task is "evaluative"; i.e., it involves the formation of an association between a particular object and reward (Ridley et al., 1999). Thus, in the example above, the pen cap was always situated over the food reward, whether presented on the right or the left, and the toy was never situated over the reward.

Over a number of trials, marmosets acquired the association, and eventually selected the "correct" object (A, the pen cap) with sufficient accuracy (90% correct) to "pass" the task and move onto the next task in the experimental design schedule.

Hippocampal task: the visuospatial discrimination task. Visuospatial discrimination tasks require a different type of learning from the visual discrimination task described above. Visuospatial tasks require "conditional" (rather than associative) learning to take place for successful completion (Ridley and Baker, 1991), because all objects are equally associated with reward, and a "rule" must be learned instead of a simple association.

Pairs of identical objects are used in this instance. For every task, two pairs of identical objects were used, and in each trial, two identical objects were seen by the marmoset: one over the left food well and the other over the right food well. For example, objects Y and Y may be identical colored key rings, whereas objects Z and Z might be identical Eppendorf tubes. On any single trial, the marmoset could see either the two key rings, or the two Eppendorf tubes. The choice of which pair was seen was again determined by a pseudorandom schedule.

Marmosets choose an object by moving it to reveal the reward (or

lack thereof) hidden underneath. The conditional rule that must be adhered to was that when objects Y and Y were seen, the reward would be hidden under the rightmost of the two identical objects; when objects Z and Z were seen, the reward would be hidden under the leftmost example of object Z. So in the example given above, when the key rings were seen, food was under the right key ring, and when the Eppendorf tubes were seen, food was under the left tube. Marmosets had to reach a criterion of 90% correct to pass the task and move onto the next.

Study Design. The first stage of the study was to "shape" the marmosets, i.e., to take experimentally naive animals and train them to respond to objects presented in the WGTA. All animals were trained with an identical series of tasks: two perceptually simple visual discrimination tasks; the first taken to a criterion of 90 correct of 100 consecutive trials (90/100) and the second to a criterion of 27 correct of 30 consecutive trials (27/30). This was followed by two visuospatial discrimination tasks; the first to a criterion of 90/100 and the second to a criterion of 27/30. Last, animals learned two perceptually complex (black object) visual discrimination tasks to a criterion level of 27/30 in both cases.

Once these tasks had been successfully mastered, marmosets were assigned to either the perceptually complex visual discrimination study or the visuospatial discrimination study, such that there was an equal spread of learning ability across the two studies [by balancing learning scores (number of trials to achieve criterion) between groups]. Other marmosets, after completing the same training, were assigned to either the surgery or the control group, in such a way that there was an equal spread of learning ability between the two groups before surgery took place.

In all experiments, the marmosets learned a series of consecutive tasks using new objects for each task. All tasks were presented in the same order, such that interference between stimuli was kept identical for all monkeys. Treatments were given daily for the duration of learning task (as described above). Learning scores (the number of trials before the 27/30 correct criterion was reached) were averaged for each animal under each treatment to balance for practice effects across the tasks. At the end of the study, mean data for each animal under each treatment were analyzed, and the control group was compared with the lesion group.

Data Analysis

A repeated measures ANOVA was used to analyze the data, using a Statview II software package on an Apple Macintosh computer. The mean learning scores for each marmoset under each treatment provided the raw data. Post hoc analyses included Dunnett's t test (for comparing various treatments to control/vehicle data) and Scheffé's F test (for comparing different treatments to one another). In all cases, $p < 0.05$ indicated a significant difference.

All animal procedures were approved by the Animal Care Committees of Princeton University and Wyeth Research and conformed with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Materials

All chemicals were of analytical grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI). 8-OH-DPAT was purchased from Sigma/RBI. Lecozotan was synthesized by Chemical Sciences (Wyeth Research, Princeton, NJ).

Results

In Vitro Profile and Structure

The chemical structure of lecozotan is shown in Fig. 1. Lecozotan displayed high-affinity binding at the cloned human 5-HT$_{1A}$ receptor ($K_i = 1.6 \pm 0.3$ and 4.5 ± 0.6 nM) using [^{3}H]8-OH-DPAT as the 5-HT$_{1A}$ agonist radioligand and [^{3}H]WAY-100635 as the 5-HT$_{1A}$ antagonist radioligand, re-
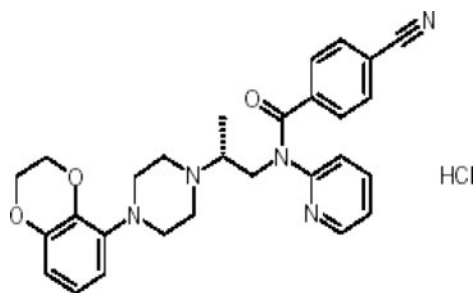
Fig. 1. Chemical structure of lecozotan.

spectively (Table 1). Lecozotan was selective for the 5-HT$_{1A}$ receptor over other monoamine receptors that have previously been shown to be cross-reactive with the aryl-piperazine pharmacophore. A Nova Screen profile revealed that greater than 50% displacement was only recorded at the highest concentration of 10,000 nM at 22 sites, indicating that lecozotan demonstrates significant selectivity for the 5-HT$_{1A}$ receptor. Lecozotan did not exhibit significant activity (>50% inhibition at any concentration) at any of the other 61 binding sites that were evaluated at that concentration except for dopamine D$_4$ (inhibition 63%). Most notably, the compound was not active at various α-adrenergic (1A, 1B, 2A, 2B, and 2C), β-adrenergic (1 and 2), adenosine (1–3), dopamine (1, 2, 3, and 5), histamine (1–3), muscarinic (1–5), or serotonin (1B, 1D, 2A, 2C, and 3–7) receptors.

In a clonal CHO cell line expressing the human 5-HT$_{1A}$ receptor, lecozotan antagonized the ability of 8-OH-DPAT to inhibit forskolin-stimulated cAMP production. In this study, the response to 10 nM 8-OH-DPAT, representing a submaximal concentration, was evaluated in the presence of increasing concentrations of antagonist. The calculated IC$_{50}$ for lecozotan was 25.1 ± 5.3 nM (Table 1). These results were corroborated using a [^{35}S]GTPγS assay where lecozotan also blocked the agonist effects of 8-OH-DPAT (IC$_{50}$ = 36.7 ± 0.74 nM). Lecozotan alone did not induce any agonist-like activity under any assay conditions.

Lecozotan Attenuates 8-OH-DPAT-Induced Changes in Hippocampal 5-HT Levels without Having Basal Effects: In Vivo Microdialysis

Lecozotan (3.0 mg/kg s.c.) had no effect on basal levels of 5-HT in the hippocampus of conscious rats when compared with vehicle-treated animals (Fig. 2). Treatment with 8-OH-DPAT (0.3 mg/kg s.c.) induced a significant ($F_{1,24}$ = 7.9; p = 0.0089) decrease in extracellular levels of 5-HT. Pretreat-
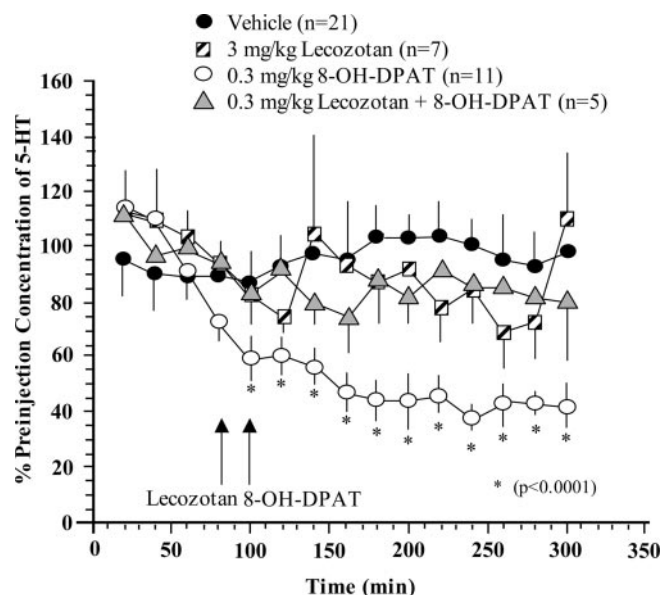
TABLE 1

In vitro binding and functional profile of lecozotan

Lecozotan was evaluated in a broad Nova Screen binding profile, where it was determined that the compound was >100-fold-selective with respect to 63 neurotransmitter, receptor, reuptake, ion channel, and enzyme sites.

Compound	K_i					IC$_{50}$	
	5-HT$_{1A}$	α1	D$_2$	D$_3$	D$_4$	cAMP	GTPγS
	nM					*nM*	
Lecozotan (nM)	1.6 ± 0.3[a]	248	1548	320	98	25.1 ± 5.3	36.7 ± 0.74
	4.5 ± 0.6[b]						

α1, subtype 1 of the α-adrenergic receptor; D$_2$, subtype 2 of the dopamine receptor; D$_3$, subtype 3 of the dopamine receptor; D$_4$, subtype 4 of the dopamine receptor.
[a] [^{3}H]8-OH-DPAT.
[b] [^{3}H]WAY-100635.

Fig. 2. Lecozotan administered s.c. significantly ($F_{2,21}$ = 19.49; p < 0.0001) antagonized the decrease of hippocampal extracellular 5-HT levels induced by 8-OH-DPAT in rats. Data are expressed as mean ± S.E.M. of percentage of preinjection baseline values. Lecozotan had no effect up to 3 mg/kg when administered alone.

ment with lecozotan (0.3 mg/kg s.c.) produced a significant ($F_{2,21}$ = 19.49; p < 0.0001) and complete attenuation of the 8-OH-DPAT-induced decrease in hippocampal 5-HT.

Lecozotan Competitively Blocks the Inhibition of Dorsal Raphe Neuronal Firing Induced by 8-OH-DPAT

8-OH-DPAT (0.025 mg/kg s.c.) produced significant inhibition of dorsal raphe neuronal (DRN) firing in the anesthetized rat (Fig. 3). At a dose of 0.3 mg/kg s.c., lecozotan produced no change in neuronal firing. The firing rates (mean action potentials per second ± S.E.M.) at baseline and after lecozotan treatment were 1.07 ± 0.25 and 1.12 ± 0.28, respectively (not shown). Pretreatment with lecozotan (0.3 mg/kg s.c.) significantly (p < 0.001) antagonized the inhibitory effects of 8-OH-DPAT (0.025 to 0.1 mg/kg s.c.). Cumulative doses up to 0.625 mg/kg of 8-OH-DPAT were needed to restore inhibition of neuronal firing to levels equal to those seen in the absence of lecozotan.

Lecozotan Antagonizes Fixed Ratio Operant Responding in Rats

8-OH-DPAT (s.c.) produced dose-dependent decreases in response rate in all rats (ED$_{50}$ = 0.18 mg/kg; 95% CI = 0.15–0.22 mg/kg). Pretreatment with 0.3 mg/kg lecozotan s.c. produced an approximate 6-fold rightward shift of the 8-OH-DPAT dose-effect curve, with the ED$_{50}$ value for 8-OH-DPAT increasing to 1.08 mg/kg (Fig. 4A). Pretreatment with 1.0 and 3.0 mg/kg lecozotan s.c. produced 12- and 20-fold rightward shifts of the 8-OH-DPAT dose-effect curve, respectively, with the ED$_{50}$ value for 8-OH-DPAT increasing to 3.68 mg/kg (95% CI = 2.35–5.77 mg/kg) at the higher dose. Pretreatment with 3, 10, and 30 mg/kg lecozotan p.o. produced approximately 3-, 6-, and 9.5-fold rightward shifts of the 8-OH-DPAT dose-effect curve, respectively (Fig. 4B). At the highest dose (30 mg/kg p.o.), the ED$_{50}$ value for 8-OH-DPAT increased approximately 10-fold to 2.08 mg/kg (95% CI = 1.33–3.20 mg/kg). These results suggest that there is a 10- to 30-fold
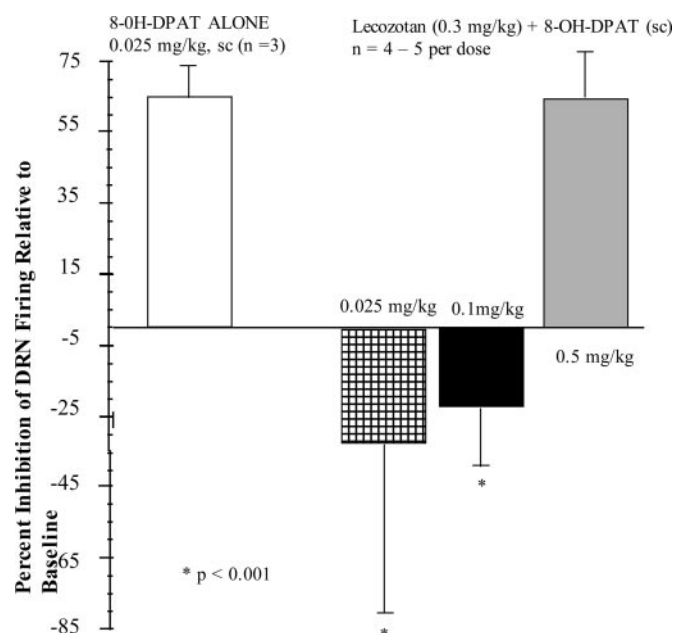
Fig. 3. Effect of lecozotan on 8-OH-DPAT-induced inhibition of DRN firing in the anesthetized rat. After lecozotan pretreatment (0.3 mg/kg), the dose of 8-OH-DPAT needed to demonstrate comparable inhibition increased to a cumulative dose of 0.625 mg/kg. ∗, $p < 0.001$ vs. 8-OHD-PAT alone.

separation between the effects of subcutaneously and orally administered lecozotan.

In an experiment to investigate the pharmacodynamic time course of antagonism, it was determined that significant ($p < 0.05$) blockade of 8-OH-DPAT by lecozotan was present as long as 4 h after administration of lecozotan (1 mg/kg s.c.), data not shown. A single dose of 0.3 mg/kg 8-OH-DPAT s.c. decreased the response rate to 0.74 responses/s in the first component of the session (10 to 20 min after administration of 8-OH-DPAT). Rates of responding gradually returned to baseline rates across the 1-h session with a response rate of 2.33 responses/s in the third component of the session (50 to 60 min after administration of 8-OH-DPAT). After a 2- or 4-h pretreatment with lecozotan (1.0 mg/kg s.c.), the decreases in

response rates observed after 0.3 mg/kg 8-OH-DPAT alone were completely antagonized for the entire session, with response rates greater than 2 responses/s in all three response components.

Lecozotan Antagonizes Fixed Ratio Operant Responding in Squirrel Monkeys

8-OH-DPAT i.m. produced a dose-dependent decrease in response rates in all monkeys (ED_{50} value of 0.07 mg/kg; 95% CI = 0.04–0.11 mg/kg). Lecozotan administered at doses of 0.01 and 0.1 mg/kg i.m. produced dose-dependent rightward shifts of the dose-effect curve for 8-OH-DPAT (Fig. 5A). At the highest dose (0.1 mg/kg), the ED_{50} value for 8-OH-DPAT was shifted approximately 17-fold to 1.21 mg/kg (95% CI = 0.45–3.25 mg/kg). Similar shifts in the 8-OH-DPAT dose-effect curves were produced by the oral administration of lecozotan at doses of 0.1, 0.3, and 1.0 mg/kg (Fig. 5B). At the highest dose (1.0 mg/kg), the ED_{50} value for 8-OH-DPAT was shifted approximately 10-fold to 0.68 mg/kg (95% CI = 0.27–1.72 mg/kg).

Lecozotan Blocks the Discriminative Stimulus Effects of 8-OH-DPAT in Pigeons

Once animals were trained to the stimulus cue, animals responded almost exclusively (>98%) on the drug-appropriate key after an injection of 0.1 mg/kg 8-OH-DPAT and on the saline-appropriate key (<2% drug-key responses) after an injection of saline (Fig. 6). Subsequently, lecozotan was assessed for its ability to block the stimulus cue evoked by an acute injection of 8-OH-DPAT. Lecozotan was administered as a pretreatment (0.01–1 mg/kg i.m.) to the training dose of 0.1 mg/kg 8-OH-DPAT i.m. Lecozotan dose dependently decreased the percentage of responses on the 8-OH-DPAT appropriate key, consistent with its actions as a 5-HT_{1A} receptor antagonist (Fig. 6). Response completely shifted to the saline-appropriate key after administration of 1.0 mg/kg lecozotan. Response rates were unaffected by lecozotan when given alone at doses of 0.01 to 1.0 (data not shown).
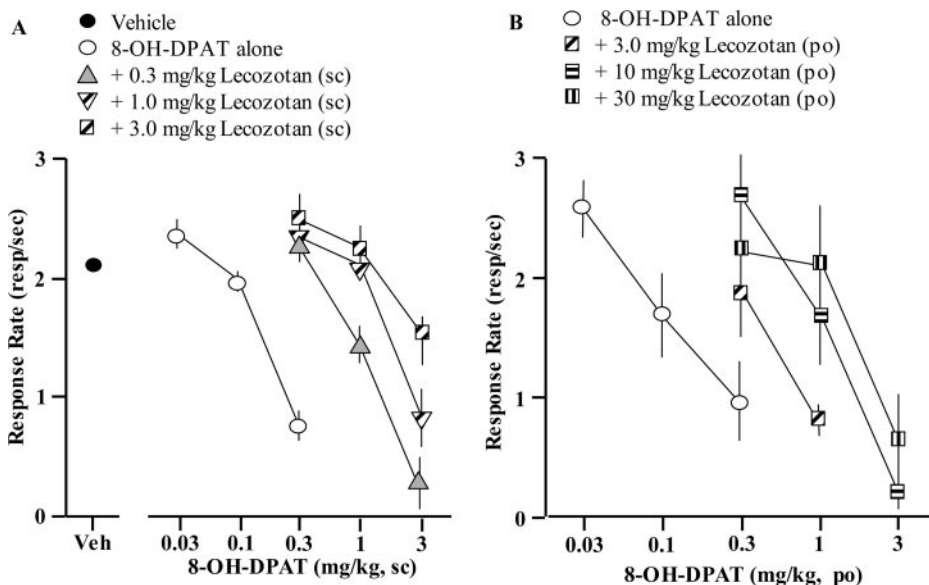

Fig. 4. 8-OH-DPAT (s.c.) produced decreases in fixed ratio operant response rates in all rats. Pretreatment with lecozotan administered s.c. (A) and p.o. (B) shifted the 8-OH-DPAT dose effect curve to the right. Values are means ± S.E.M.
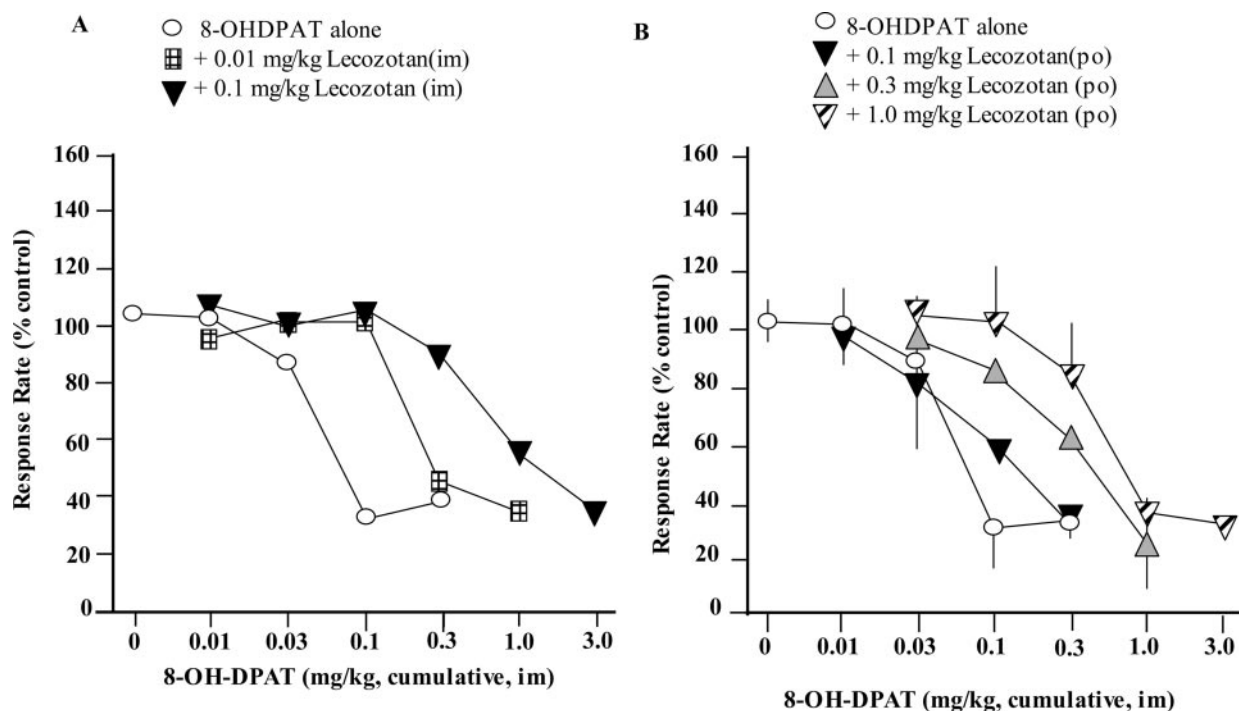
Fig. 5. 8-OH-DPAT (i.m.) produced decreases in fixed ratio operant response rates in all squirrel monkeys. Pretreatment with lecozotan administered i.m. (A) and p.o. (B) shifted the 8-OH-DPAT dose effect curve to the right. Values are means ± S.E.M.

Lecozotan Antagonizes the 5-Methoxy-dimethyltryptamine-Induced Syndrome in the Rat

The 5-HT syndrome is mediated by postsynaptic 5-HT$_{1A}$ receptors and is considered a model of central 5-HT$_{1A}$ receptor stimulation. The 5-HT syndrome for these studies was induced by 5-MeODMT at a dose of 5.6 mg/kg i.p. Lecozotan (10 mg/kg) or vehicle (1 ml/kg) were given p.o. once daily for seven consecutive days to rats (Tables 2 and 3). Twenty-four hours after the last dose, the antagonist potency was determined using the 5-MeODMT-induced syndrome. ID$_{50}$ was 4.26 mg/kg (95% CI = 2.23–8.13) after chronic lecozotan administration, which did not differ significantly from the

acute potency or the potency after chronic vehicle (4.92 mg/kg; 95% CI = 2.70–9.00). Lecozotan exhibited no agonist activity by itself when administered up to 17 mg/kg. Thus, chronic lecozotan treatment did not alter the antagonist potency in this model.

In separate experiments, the agonist potency of 5-MeODMT was evaluated 24 h after the last dose. An ED$_{50}$ value of 1.83 mg/kg (95% CI = 1.37–2.43) was calculated after i.p. administration of chronic vehicle. An ED$_{50}$ value of 1.68 mg/kg (95% CI = 1.00–2.82) was observed after chronic i.p. administration of lecozotan (10 mg/kg). Thus, chronic lecozotan treatment did not alter the sensitivity of 5-HT$_{1A}$ receptors.

Effects of Lecozotan on Basal and Stimulated Glutamate Release from the Dentate Gyrus

Lecozotan produced a significant ($F_{2,17} = 4.52$; $p = 0.027$) augmentation of K$^+$-induced increase in extracellular glutamate concentrations within the dentate gyrus of the rat.
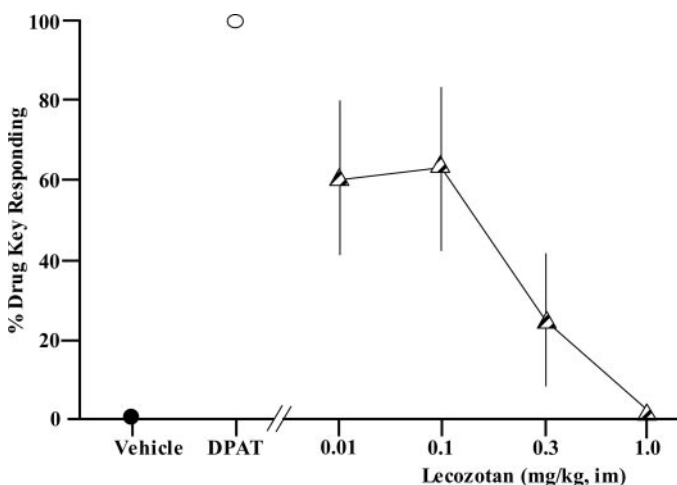

Fig. 6. In a discriminative stimulus effects study using pigeons, lecozotan (0.01–1.0 mg/kg) dose dependently decreased the percentage of responses on the 8-OH-DPAT-appropriate key, consistent with its actions as a 5-HT$_{1A}$ receptor antagonist. Values are means ± S.E.M. percentage of drug key responding. 8-OH-DPAT (i.m.) training dose was 0.1 mg/kg. Saline was used as a vehicle.
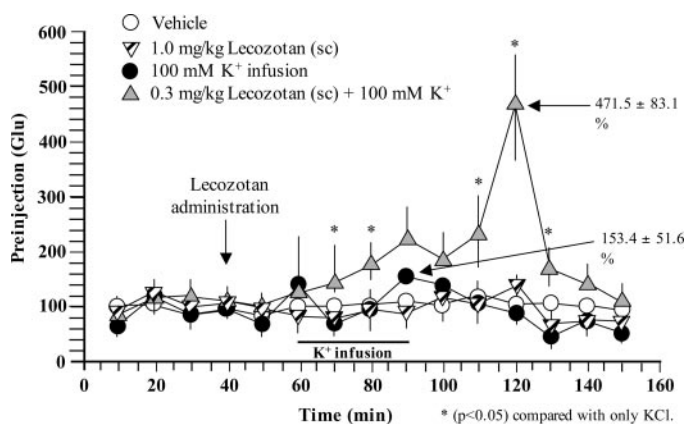
TABLE 2
Potency of lecozotan to antagonize 5-MeODMT-induced syndrome after chronic lecozotan

Chronic Treatment	ID$_{50}$ (95% CI)
	mg/kg
Vehicle (1 ml/kg p.o.)	4.92 (2.70–9.00)
Lecozotan (10 mg/kg p.o.)	4.26 (2.23–8.13)

TABLE 3
Potency of 5-MeODMT to induced syndrome after chronic lecozotan

Chronic Treatment	ED$_{50}$ (95% CI)
	mg/kg
Vehicle (1 ml/kg i.p.)	1.83 (1.37–2.43)
Lecozotan (10 mg/kg i.p.)	1.68 (1.00–2.82)

Fig. 7. Effects of lecozotan on basal and K^+-stimulated glutamate levels in the dentate gyrus of rats. Data are expressed as means $\pm$ S.E.M. of percentage of preinjection levels of glutamate; $n = 6$ to 8 per study group. Lecozotan significantly ($F_{2,17} = 4.5$; $p = 0.027$) augmented the K^+-stimulated increases in extracellular glutamate within the dentate gyrus reaching a maximum value of $471.5 \pm 83.1\%$ at 120 min ($p = 0.012$). Asterisk ($*$) denotes statistical ($p < 0.05$) difference between K^+-stimulated versus lecozotan + K^+-stimulated responses.

Administration s.c. of lecozotan (1.0 mg/kg) alone produced no change in basal levels of glutamate within the dentate gyrus (Fig. 7). Infusion of aCSF containing 100 mM KCl produced a small but nonsignificant ($p = 0.053$) increase in extracellular glutamate levels, reaching a maximum value of $153.4 \pm 51.6\%$ of preinfusion control levels. Pretreatment s.c. with a single dose of lecozotan (0.3 mg/kg) produced a significant potentiation of K^+-induced increases in extracellular glutamate with a maximum value of $471.5 \pm 83.1\%$ of preinfusion levels ($p = 0.012$).

Effects of Lecozotan on Basal and Stimulated Acetylcholine Release from the CA1 Region of the Hippocampus

Lecozotan administered s.c. at a dose of 1 mg/kg had a small but significant effect on basal acetylcholine levels ($146 \pm 5\%$) in the CA1 region of the hippocampus compared with vehicle ($125 \pm 7\%$). When a dose of 1 mg/kg lecozotan was administered s.c. before a 10-min infusion of KCl (100 mM), extracellular acetylcholine levels increased ($p < 0.05$) to $275 \pm 27\%$ over basal levels (Fig. 8).
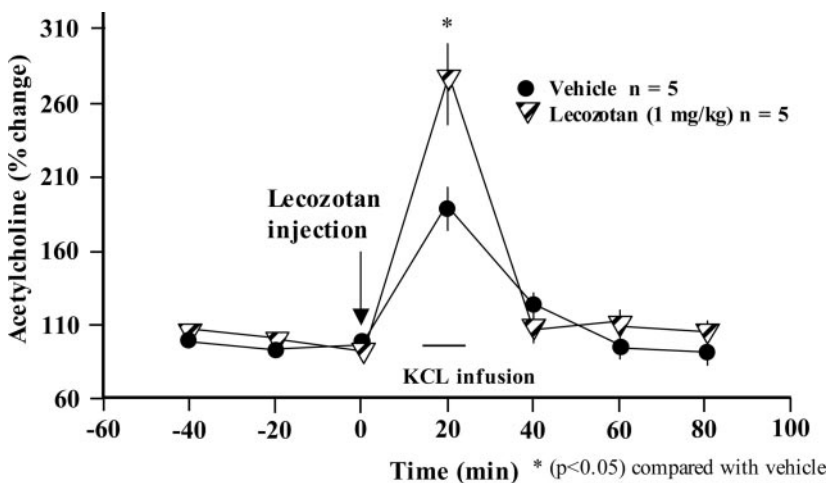
Lecozotan Enhances Cognitive Performance in the Aged Rhesus Monkey

Vehicle treatment was associated with a significant ($F_{3,40} = 35.7$; $p < 0.001$) delay-dependent reduction in task accuracy that approximated the target accuracies indicated under *Materials and Methods*. Figure 9A shows the composite data set for the effects of lecozotan i.m. on DMTS performance efficiency. Statistical analysis of the data revealed no significant effect of treatment alone ($F_{4,40} = 1.03$; $p = 0.40$), and no significant effect of treatment's interaction with delay interval ($p = 0.49$). There also were no significant effects of treatment for sessions run 24 h after drug administration (data not shown). On average, however, improvement in task performance efficiency was maintained for the 0.3 and the 1 mg/kg doses for trials associated with short delay intervals.

The optimal (best) dose was defined as the dose that evoked the greatest task improvement independent of delay interval. Three subjects received overall (all 96 trials) benefit (overall task accuracy greater than respective vehicle level) from two of the five doses. One subject received benefit from three of the five doses, and one subject received benefit from only one dose. Therefore, none of the subjects could be considered a nonresponder under the conditions of the experiment. Most of the average task improvement was noted in the middle of the dose-response relationship: two animals received most improvement from the 1 mg/kg dose, and the other three animals' best doses were 0.1, 0.3, and 1 mg/kg. The average best dose for the study group was 1.1 mg/kg.

To reduce the likelihood of false positives, the best dose was repeated on one additional occasion. Both sessions were averaged, and the data are presented in Fig. 9B. There was a significant effect of drug treatment, independent of delay interval ($F_{1,44} = 7.31$; $p = 0.01$). When improvement in task efficiency was calculated as the percentage increase from baseline levels of performance, after best dose, accuracy increased by 16.5% of control for trials associated with short delay intervals. Although the treatment times delay effect was not statistically significant, the improvement in accuracy associated with short delay trials accounted for 45% of the total task improvement.

In addition to task accuracies, two response latencies were measured: one to initiate the trial and one to initiate a choice. Drug treatment did not significantly affect ($F_{2,40} = 2.19$; $p =$

Fig. 8. Lecozotan (1.0 mg/kg s.c.) produced a significant potentiation of 100 mM K^+-induced increases in extracellular acetylcholine levels in the CA1 region of the hippocampus. Values are means $\pm$ S.E.M. Asterisk ($*$) indicates significant difference ($p < 0.05$) compared with vehicle.
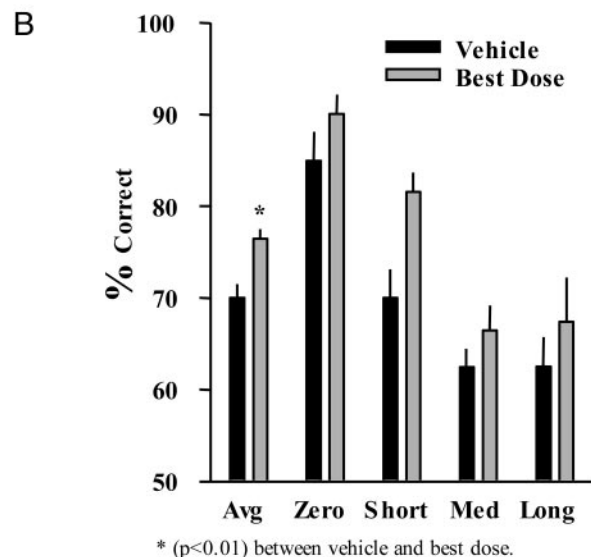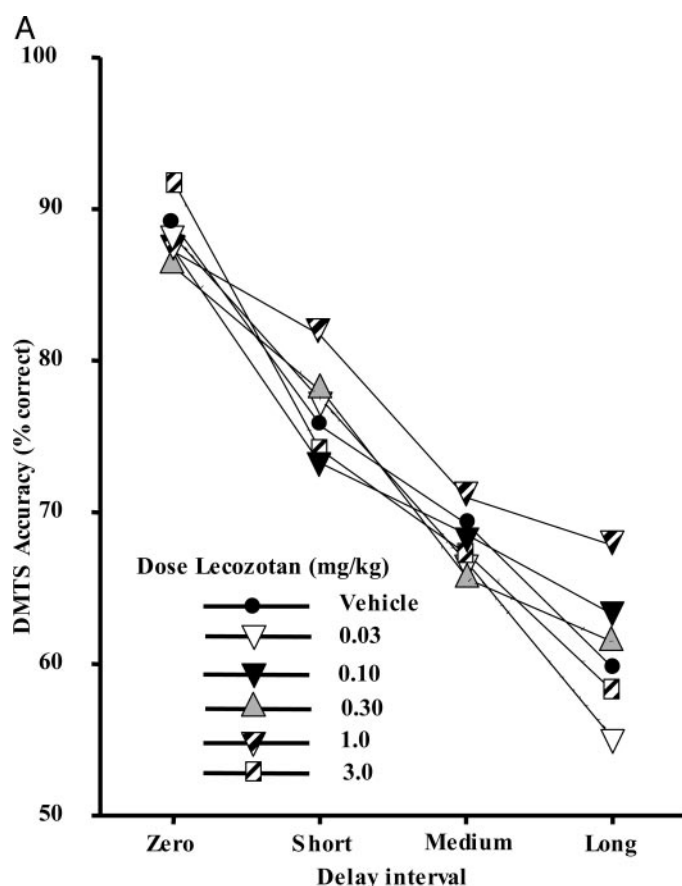
Fig. 9. A, lecozotan enhances cognitive function in the aged rhesus monkey as assessed by DMTS performance efficiency. Testing was initiated 30 min after a single dose administration. Statistical analysis of the data revealed no significant effect of treatment alone ($p = 0.40$), and no significant effect of treatment's interaction with delay interval ($p = 0.49$). B, improvement in DMTS accuracy produced by the individualized best dose of lecozotan. These data represent the average of the best dose session determined from the dose-response series and a separate series in which only the best dose was administered. There was no significant difference between the data sets obtained for these two series ($t = 0.44$; $p = 0.66$). For the combined sessions, there was a significant effect of drug treatment relative to vehicle that was independent of delay interval. Values are means ± S.E.M. Asterisk (∗) indicates significant difference ($p = 0.01$) compared with vehicle. Avg, average; Med, medium.

0.13) the duration of either response latency (data not shown).

Lecozotan Reverses Cognitive Deficits Associated with Glutamatergic Dysfunction or Cholinergic Lesions in Marmosets

Acquisition of Visuospatial Discrimination Tasks after VDB Lesion Surgery. VDB-lesioned animals' mean learning scores indicate significant impairment ($p < 0.05$) compared with nonlesioned (control) animals (Fig. 10). Lecozotan (1 and 2 mg/kg i.m.) had no significant effect in the control animals at either dose. In lesioned animals, lecozotan-treated (1 mg/kg) animals had learning deficits significantly greater ($p < 0.05$) than similarly treated nonlesioned animals. Lecozotan at 2 mg/kg, however, completely abolished the lesion-induced cognitive deficit. Mean learning scores after lecozotan 2 mg/kg treatment were not significantly different from learning scores of nonlesioned animals treated with 2 mg/kg lecozotan, and significantly ($p < 0.05$) lower than the scores of saline- and lecozotan-treated (1 mg/kg) lesioned animals. Overall, data suggest that lecozotan (2 mg/kg i.m.) completely reverses a hippocampal cognitive deficit induced by cholinergic lesion.

Acquisition of Perceptually Complex Visual Discrimination Tasks. Dizocilpine (0.015 mg/kg) caused a significant ($p < 0.001$) deficit in the marmosets' ability to acquire perceptually complex visual discrimination tasks compared with saline treatment (Fig. 11A). Lecozotan (2 mg/kg) alone had no significant effect on mean learning score
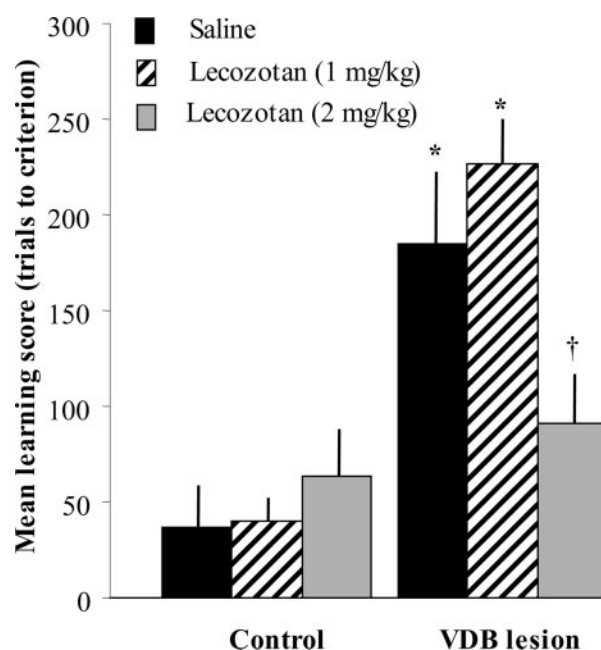
Fig. 10. Deficits in acquisition of a hippocampal task (visuospatial discrimination) in marmosets were induced by a VDB lesion. Deficits were abolished by lecozotan at 2 mg/kg but not at 1 mg/kg. Values are mean learning scores ± S.E.M. Asterisk (∗) indicates that learning scores for VDB-lesioned marmosets were significantly greater than the scores of nonlesioned animals when saline (vehicle) or 1 mg/kg lecozotan was administered during task acquisition ($p < 0.05$). Dagger (†) indicates that learning scores for VDB-lesioned marmosets were significantly lower (i.e., acquisition was faster) when these lesioned marmosets were treated with 2 mg/kg lecozotan, compared with when they were treated with saline or 1 mg/kg lecozotan ($p < 0.05$). Additionally, VDB-lesioned marmosets acquired the task as quickly as unlesioned marmosets when they were treated with 2 mg/kg lecozotan ($p > 0.05$, N.S.).
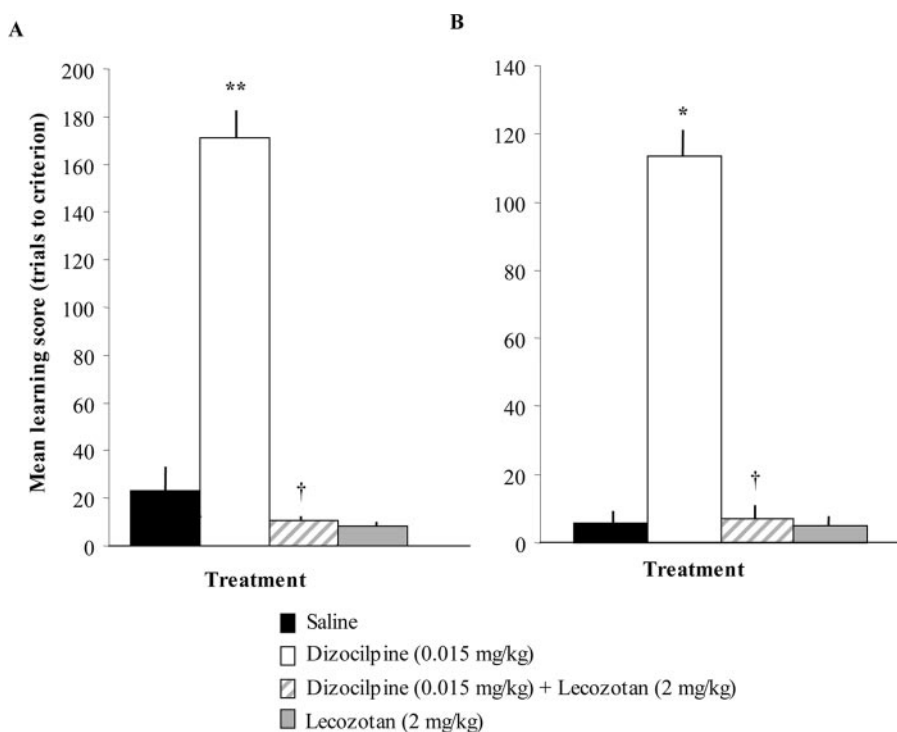
A

B

Fig. 11. A, deficits in acquisition of a hippocampal task in marmosets induced by dizocilpine (NMDA antagonist) are completely reversed by lecozotan (i.m.) as assessed using complex visuospatial discrimination. Marmosets under dizocilpine treatment had significant (**∗∗**, $p < 0.001$) deficits in learning scores compared with their scores under saline treatment. Lecozotan (2.0 mg/kg) significantly (†, $p < 0.001$) reversed the dizocilpine-induced deficits. Values are means ± S.E.M. B, deficits in acquisition of a neocortical task in marmosets induced by dizocilpine (NMDA antagonist) are completely reversed by lecozotan (2 mg/kg) as assessed using perceptually complex visual discrimination. Marmosets under dizocilpine treatment had significant (∗, $p < 0.05$) deficits in learning scores compared with their scores under saline treatment. Lecozotan (2.0 mg/kg) significantly (†, $p < 0.05$) reversed the dizocilpine-induced deficits. Values are means ± S.E.M.

compared with saline treatment. When lecozotan (2 mg/kg) was given concurrently with dizocilpine, the dizocilpine-induced deficit in task acquisition was abolished ($p < 0.001$). Overall, data suggest that lecozotan i.m. reverses cognitive deficits induced by glutamatergic blockade in a cortically mediated task.

Acquisition of Visuospatial Discrimination Tasks. Dizocilpine at 0.015 mg/kg caused a significant ($p < 0.05$) deficit in the marmosets' ability to acquire visuospatial discrimination tasks compared with saline treatment (Fig. 11B). Lecozotan at 2 mg/kg had no significant effect on mean learning score compared with saline treatment. When 2 mg/kg lecozotan was given concurrently with dizocilpine, the dizocilpine-induced deficit in task acquisition was abolished ($p < 0.05$). Overall, data suggest that lecozotan i.m. completely reverses a dizocilpine-induced cognitive deficit in hippocampally mediated tasks.

Discussion

In-depth pharmacological studies in this report demonstrate that lecozotan is a novel 5-HT$_{1A}$ receptor antagonist with intriguing procognitive properties. In vitro and in vivo assays designed to test the intrinsic activity of lecozotan demonstrate that it is a potent and selective 5-HT$_{1A}$ receptor antagonist. This inclusive series of studies has determined the intrinsic activity of lecozotan in models indicative of pre- versus postsynaptic 5-HT$_{1A}$ receptor function. Molecules that possess intrinsic activity at the presynaptic 5-HT$_{1A}$ autoreceptor lead to decreases in serotonergic cell firing and subsequent reductions in 5-HT levels in terminal regions. Lecozotan demonstrated an antagonist profile, because administration of relatively high doses resulted in no change in either extracellular 5-HT levels in terminal projection areas of the serotonergic cell bodies or any change in serotonergic neuronal firing in the DRN. Instead, lecozotan completely

antagonized 8-OH-DPAT-induced decreases in hippocampal 5-HT, indicating that lecozotan seemed to act as a 5-HT$_{1A}$ receptor antagonist in vivo. These data contrast with those observed for the 5-HT$_{1A}$ receptor partial agonists BMY-7378, NAN-190, and buspirone, which can antagonize postsynaptic 5-HT$_{1A}$-mediated responses but act as agonists at the somatodendritic 5-HT$_{1A}$ autoreceptors (Yocca, 1990; Greuel and Glaser, 1992).

Lecozotan demonstrated potent in vivo antagonist activity in rodents, nonhuman primates, and pigeons. Pharmacological studies using fixed ratio responding in rats and squirrel monkeys demonstrated that the compound is a competitive antagonist. Drug discrimination studies in pigeons revealed that lecozotan blocked the discriminative stimulus effects of the prototypical 5-HT$_{1A}$ receptor agonist 8-OH-DPAT, but it did not substitute for the discriminative stimulus effects of 8-OH-DPAT. Chronic administration of lecozotan did not seem to alter the sensitivity of 5-HT$_{1A}$ receptors in that there was no significant shift in the potency of an agonist to induce serotonin syndrome in male rats after continuous administration of lecozotan. Together, these data show lecozotan to be a potent, selective, and orally bioavailable competitive 5-HT$_{1A}$ receptor antagonist in vivo that produces no evidence of tolerance to its antagonist effects or alters the sensitivity of 5-HT$_{1A}$ receptors.

Alzheimer's disease is a neurodegenerative disorder characterized by various deficits in neurotransmitter function. It is widely thought that loss of these functions contributes to the loss in memory that is the hallmark of this disease. The hippocampus is one of the major brain structures shown to play a critical role in both mnemonic and cognitive function (Eichenbaum et al., 1992; Jarrard, 1993). Degeneration of the pyramidal cells in the corticohippocampal and corticocortical systems is one of the earliest changes in AD (Braak and Braak, 1991). Whereas many studies have concentrated on the cholinergic deficits observed in this disease, glutamater-

gic dysfunction in Alzheimer's disease has received increasing interest as an alternative to the well established cholinergic hypothesis (Maragos et al., 1987; Proctor et al., 1988; Francis et al., 1993; Myhrer, 1993).

Anatomical data indicate that 5-HT$_{1A}$ receptors might control the excitability and propagation of information transmitted by the pyramidal cells to subcortical structures along glutamate pathways (Czyrak et al., 2003). Serotonin has been shown to exhibit inhibitory effects on the glutamatergic projections of the brain, in particular those of the cortex (Dijk et al., 1995; Grunschlag et al., 1997) and hippocampus (Schmitz et al., 1995; Matsuyama et al., 1996). The cellular mechanisms underlying these processes seem to involve activation of K$^+$ conductance (Grunschlag et al., 1997) and/or inhibition of presynaptic Ca^{2+} entry (Schmitz et al., 1995; Strosznajder et al., 1996). Other data suggest that 5-HT$_{1A}$ receptor antagonists can inhibit the tonic hyperpolarizing action of serotonin on pyramidal neurons in both the cortex and hippocampus (Araneda and Andrade, 1991; Van den Hooff and Galvan, 1992). Thus, it is possible that 5-HT$_{1A}$ receptor antagonists may improve cognition by removing the inhibitory effects of endogenous serotonin on pyramidal neurons and enhancing the glutamatergic activation in both the cortex and hippocampus. An important aspect of this hypothesis is supported by the suggestion that a functionally hyperresponsive serotonin system in AD may contribute to cognitive disturbances (McLoughlin et al., 1994).

To test the hypothesis that a 5-HT$_{1A}$ receptor antagonist would enhance glutamatergic neurotransmission, we used lecozotan to examine excitatory transmission under basal and stimulated conditions within the terminal regions of the perforant pathway (i.e., granular layer of the dentate gyrus) using in vivo microdialysis. Lecozotan alone produced no change in basal levels of glutamate within the dentate gyrus, but it induced significant augmentation of dentate gyrus glutamate levels when administered in the presence of an infusion of K$^+$ in a way similar to that observed previously with WAY-101405 (Schechter et al., 2000). Furthermore, these in vivo data confirm the in vitro observations of Matsuyama et al. (1996), who demonstrated that NAN-190 could augment NMDA-induced increases in glutamate in the guinea pig dentate gyrus. Serotonergic modulation of glutamatergic neurotransmission via the 5-HT$_{1A}$ receptor has also been observed in other brain regions (Dijk et al., 1995; Schmitz et al., 1995). Thus, lecozotan-enhanced stimulated release of glutamate in the hippocampus supports the contention that 5-HT$_{1A}$ receptor antagonists can modulate stimulated glutamate release as part of the mechanism related to enhancing cognitive function.

The "cholinergic hypothesis", which claims that AD is primarily the result of substantial cholinergic abnormalities, has received widespread attention in the development of therapeutic strategies directed at this psychopathological illness (Bartus, 2000). A major feature of the neuropathology of AD is the degeneration of the cholinergic magnocellular neurons in the basal forebrain. A high density of 5-HT$_{1A}$ binding sites has been reported in the region of cholinergic cell bodies within the nucleus basalis magnocellularis. WAY-100635 reverses the choice accuracy deficit in rats with α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-induced lesions of the nucleus basalis magnocellularis (Balducci et al., 2003). Our data indicated that in addition to the ability of lecozotan

to enhance stimulated glutamate efflux, the 5-HT$_{1A}$ receptor antagonist is also able to enhance stimulated acetylcholine levels and reverse cognitive deficits induced by cholinergic lesions.

Serotonin-1A receptor antagonists potentiate NMDA-induced glutamate release from pyramidal neurons. Furthermore, a 5-HT$_{1A}$ receptor antagonist has the ability to significantly elevate glutamate release when administered alone (Araneda and Andrade, 1991). Since cognitive deficits have previously been shown to be susceptible to cognition enhancing treatments in the marmoset monkey (Harder and Ridley, 2000), the use of the NMDA receptor antagonist dizocilpine is appropriate for the evaluation of putative cognitive enhancers targeted at dementia. Degeneration of the cholinergic cells of the basal forebrain is the other major feature that has long been linked to cognitive dysfunction. In our marmosets, a specific and selective lesion of the cholinergic cell bodies of the VDB eliminated the majority of the cholinergic input to the hippocampus and produced the expected cognitive deficit in visuospatial learning. Because the deficit obtained has been shown to be susceptible to cognitive-enhancing agents, this "model" is also appropriate for evaluating dementia treatments. The fact that the 5-HT$_{1A}$ antagonist lecozotan was able to overcome both a VDB lesion and the effects of an NMDA receptor antagonist compound suggests that it is a potentially valuable cognitive-enhancing agent. In addition, WAY-100635 antagonizes the extinction of recognition memory and counteracts scopolamine-induced performance deficits in the normal rat (Pitsikas et al., 2003). WAY-101405, an orally active 5-HT$_{1A}$ antagonist, can reverse the deficits induced by MK-801 in rats in the radial arm maze (Boast et al., 1999). In contrast to the cognitive-enhancing properties of 5-HT$_{1A}$ antagonists, the prototypical 5-HT$_{1A}$ agonist 8-OH-DPAT can impair passive avoidance performance in the rat (Santucci and Shaw, 2003; Winsauer et al., 1999). WAY-100635 has been shown to reverse the cognitive deficits in marmosets induced by a fornix lesion and reverses the cognitive deficits induced by the glutamatergic antagonist MK-801 and the muscarinic antagonist scopolamine as tested using visuospatial and visual discrimination tasks in marmosets (Harder et al., 1996; Harder and Ridley, 2000). These studies, which tested different aspects of cognition and cognitive impairment induced by various neurochemical and neuroanatomic insults, strongly suggest that 5-HT$_{1A}$ receptor antagonism is a viable drug strategy for restoring the multiple deficits associated with AD, and as such, will represent a novel and unique treatment approach. This potential therapeutic utility is further supported by the data in aged monkeys. Here, the effects of lecozotan administration on DMTS task accuracy did not rise to statistical significance, owing primarily to individual variations in drug responsiveness. However, the dose-response study revealed that, at least on average, the 1 mg/kg dose increased accuracy, particularly during short-delay trials. Selection and readministration of the individualized best dose was consistent with the dose-response analysis in that the average best dose was 1.1 mg/kg and that there was an overall significant improvement in accuracy, which again was primarily associated with short-delay trials.

The degree of cognitive improvement induced by lecozotan in aged rhesus monkeys using DMTS, as indicated by the 16.5% increase in task accuracy above baseline, represents a

very good level of efficacy compared with other compounds tested under similar conditions in this setting (Buccafusco and Terry, 2000). The observation that on average the effect was largely relegated to trials associated with short-delay intervals suggests the possibility that lecozotan may have a selective action to improve attentional aspects of cognition (Paule et al., 1998). Further studies using behavioral paradigms more suited to assessing drug effects on attention should be used to confirm this contention. The lack of effect on task response latencies was consistent with no effect of the drug on psychomotor speed. No untoward effects of the drug were noted before or after testing. Together, these data demonstrate the efficacy of 5-HT$_{1A}$ receptor antagonists in enhancing cognitive function in both rodents and primates.

Together, the heterosynaptic nature of the effects of lecozotan imbues this compound with a novel therapeutic approach to the treatment of the cognitive deficits associated with AD, and possibly other psychopathological disease states in need of improved therapies to treat cognitive- or memory-associated loss. Lecozotan has completed phase 1 clinical trials and is continuing clinical investigation in phase 2 as an agent to reverse cognitive loss in AD.

Acknowledgments

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