The Preclinical Pharmacological Profile of WAY-132983, a Potent M1 Preferring Agonist

ADAM C. BARTOLOMEO, HERMAN MORRIS, JERRY J. BUCCAFUSCO, NANCY KILLE, SHARON ROSENZWEIG-LIPSON, MORRIS G. HUSBANDS, ANNNMARIE L. SABB, MAGID ABOU-GHARBIA, JOHN A. MOYER, and CARL A. BOAST

Central Nervous System Research/Central Nervous System Medicinal Chemistry, Wyeth-Ayerst Research, Princeton, New Jersey (A.C.B., H.M., S.R.-L., M.G.H., A.L.S., M.A.-G., J.A.M., C.A.B.); Alzheimer’s Research Center, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia (J.J.B., N.K.); and the Veterans Administration Medical Center, Augusta, Georgia (J.J.B., N.K.)

ABSTRACT

Muscarinic M1 preferring agonists may improve cognitive deficits associated with Alzheimer’s disease. Side effect assessment of the M1 preferring agonist WAY-132983 showed significant salivation (10 mg/kg i.p. or p.o.) and produced dose-dependent hyperthermia after i.p. or p.o. administration. WAY-132983 significantly reduced scopolamine (0.3 mg/kg i.p.)-induced hyperswimming in mice. Cognitive assessment in rats used pretrained animals in a forced choice, 1-h delayed nonmatch-to-sample radial arm maze task. WAY-132983 (0.3 mg/kg i.p) significantly reduced scopolamine (0.3 mg/kg s.c.)-induced errors. Oral WAY-132983 attenuated scopolamine-induced errors; that is, errors produced after combining scopolamine and WAY-132983 (to 3 mg/kg p.o.) were not significantly increased compared with those of vehicle-treated control animals, whereas errors after scopolamine were significantly higher than those of control animals. With the use of miniosmotic pumps, WAY-132983 (0.3 mg/kg/day (s.c.) WAY-132983 significantly reduced AF64A (3 nmol/3 μlateral ventricle)-induced errors. Verification of AF64A cholinotoxicity showed significantly lower choline acetyltransferase activity in the hippocampi of AF64A-treated animals, with no significant changes in the striatal or frontal cortex. Cognitive assessment in primates involved the use of pretrained aged animals in a visual delayed match-to-sample procedure. Oral WAY-132983 significantly increased the number of correct responses during short and long delay interval testing. These effects were also apparent 24 h after administration. WAY-132983 exhibited cognitive benefit at doses lower than those producing undesirable effects; therefore, WAY-132983 is a potential candidate for improving the cognitive status of patients with Alzheimer’s disease.

In brain areas associated with cognition (e.g., cortex, hippocampus), presynaptic cholinergic markers [e.g., choline acetyltransferase (ChAT), M2 receptors] are decreased in patients with Alzheimer’s disease (AD), and these decreases are correlated with poor cognitive performance (DeKosky et al., 1992). Treatments enhancing cholinergic transmission may be useful in treating AD (Bartus et al., 1982). Targeting the cholinergic system for potential AD treatment is supported by the use of cholinesterase inhibitors (ChEIs; e.g., Tacrine, Donepezil), which reduce the breakdown of acetylcholine (Gracon, 1996; Rho and Lipson, 1997) and improve cognition in patients with AD. However, the presynaptic cholinergic degeneration that occurs in AD depletes reserves of acetylcholine that ChEIs would protect, possibly limiting their use (DeKosky et al., 1992).

In contrast, postmortem analyses of the brains of patients with AD have indicated no change in postsynaptic M1 receptor density in cortex and hippocampus (Pearce and Potter, 1991). Therefore, postsynaptic M1 agonists may provide better efficacy than ChEIs (Bodick et al., 1997). However, M1 receptor/G protein coupling may be reduced in patients with AD (Ladner et al., 1995). Although the transduction efficacy of M1 receptors may be reduced, due to reduced receptor/G protein coupling, the presence of M1 receptors provides a potential target for modulation of cognition in patients with AD. In fact, arecoline, a nonselactive muscarinic agonist, improved memory in patients with mild or moderate AD (Soncrant et al., 1993). The nonselactive of arecoline does not make it attractive as a therapeutic agent because side effects related to activity at non-M1 muscarinic receptors may be limiting. In this regard, five muscarinic receptor types have been determined and are present in central and peripheral nervous systems (Bonner, 1989). M1 receptors are predominantly localized postsynaptically in cortex, hippocampus, and the gastrointestinal tract. M2 receptors are predominantly localized presynaptically on brain cholinergic neu-
rons and in cardiac tissue. M3 receptors are predominantly located in glandular tissues, especially salivary glands. M4 and M5 receptors are not as well characterized, and specific limiting side effects have not been ascribed to them. These findings suggest that selective activation of M1 receptors may improve the cognitive deficits of AD without causing the limiting side effects that may be associated with the activation of other muscarinic receptor subtypes (Avery et al., 1997).

In addition to biochemical changes in acetylcholine (ACh) occurring in AD, AD has characteristic neuropathology, specifically, amyloid plaques, and neurofibrillary tangles. Along these lines, M1 preferring muscarinic agonists may also benefit patients with AD by shunting the cleavage of amyloid precursor protein from the insoluble β-amyloid protein fragment into the soluble, nonaggregating fragment, thus reducing the deposition of amyloid (Eckols et al., 1995). Moreover, muscarinic agonists may also decrease γ-protein phosphorylation, which could lower the formation of neurofibrillary tangles (Sadot et al., 1996).

Several M1 preferring agonists have been described, and some have been tested in patients with AD. Unfortunately, many of these agents have not proved to be effective therapeutic agents [e.g., AF102B, Fisher et al., 1996; SB 202026, Loudon et al., 1997; WAL 2014 (talsaclidine), Walland et al., 1997]. Positive effects (Bodick et al., 1997) have been demonstrated for xanomeline (Shannon et al., 1994); therefore, we compared it with the M1 agonist WAY-132983 ([3R,4R]-(-)-3-(3-hexylsulfonylpyrazin-2-yloxy)-1-azabicyclo[2.2.1]heptane hydrochloride] and xanomeline (Shannon et al., 1994); therefore, we compared it with the M1 agonist WAY-132983 ([3R,4R]-(-)-3-(3-hexylsulfonylpyrazin-2-yloxy)-1-azabicyclo[2.2.1]heptane hydrochloride); Fig. 1).

In the present study, we evaluated the in vivo effects of a novel (Sabb et al., 1999), potent (EC_{50} for PI turnover = 1.1 nM; Tasse et al., 1997) M1 preferring agonist, WAY-132983, which is approximately 6-fold more potent than xanomeline. WAY-132983 and xanomeline are approximately 7- to 8-fold selective for M1 compared with M2 receptors and approximately 3-fold selective for M1 compared with M3 receptors. Unlike xanomeline, WAY-132983 shows little activity at α-adrenergic, histamine, or 5-hydroxytryptamine_{1A} receptors and does not inhibit monoamine oxidase or acetylcholinesterase.

In vivo characterization of WAY-132983 included an assessment of its ability to induce cholinergic side effects, to function as a muscarinic agonist, and to improve cholinergic deficits in animal cognition models (radial arm maze (RAM) delayed nonmatch-to-sample (DNMTS) in rats, operant delayed-match-to-sample (DMTS) in monkeys). In the RAM, cholinergic deficits were induced either by the muscarinic antagonist scopolamine or by the selective cholinergic neurotoxin AF64A. Scopolamine can disrupt cognitive performance in the RAM (Pilcher et al., 1997). AF64A impairs RAM performance after i.c.v. administration (Bartolomeo et al., 1997), which results in damage to the septohippocampal pathway (Walsh et al., 1984) without altering M1 receptor density (Thorne and Potter, 1995). Finally, primate cognition studies consisted of a visual DMTS task, using aged monkeys.

The effects of WAY-132983 were compared with those of the M1 preferring agonist xanomeline and the nonselective cholinergic agonists arecoline, pilocarpine, and oxotremorine. WAY-132983 produced some cholinergic side effects, including salivation (SALIV) and hypothermia, but did not produce chromatodacryorrhea (CHROMO), lacrimation (LACRI), or tremors (TREM). WAY-132983 antagonized scopolamine-induced hyperswimming (Symons et al., 1986), which is consistent with muscarinic agonism. Moreover, WAY-132983 improved cognition in rats and monkeys, suggesting WAY-132983 may be effective therapy for AD.

### Materials and Methods

#### Generation of Side Effects

**Animals.** Male CD rats (Sprague-Dawley derived; Charles River Breeders, Kingston, NY) weighing 200 to 250 g were housed six per cage in a colony room with a 12-h light/dark cycle, with lights on at 6 AM. The colony room was maintained at 22°C. Animals received standard laboratory chow and water ad libitum in the colony room.

**Procedures.** Animals were injected with the test compound, removed from their home cage, and observed for the display of specific overt behaviors. The trained observer was blind to the treatment condition. Special attention was focused on CHROMO (the secretion of porphyrin, a red-pigmented substance, from lacrimal glands), LACRI, SALIV, and TREM, because these are recognized as cholinergic agonist side effects. Typically, side effects were evident by 10 min after drug administration; therefore, the presence or absence of side effects was recorded at this time point for individual animals. Although these effects often persisted for longer times, at later times no animal began to demonstrate an effect that was not present by 10 min. Core body temperatures were taken rectally by inserting 6 cm of a temperature probe that was attached to a Tele-Thermometer (YSI Inc., Yellow Springs, OH). Temperatures were recorded immediately before drug administration (baseline) as well as at various time points after drug administration. To condense the amount of data, peak hypothermia was reported. Peak hypothermia is the mean core temperature that is at the greatest decrease from the mean core temperature of the saline-injected animals. The time that peak hypothermia occurred was reported as well as the duration of measured hypothermia (versus saline-treated control animals). In addition to WAY-132983 (3, 10, or 30 mg/kg i.p. or p.o.) and xanomeline (0.3, 3, 10, or 30 mg/kg i.p. and 3, 10, or 30 mg/kg p.o.), several well characterized nonselective muscarinic agonists (oxotremorine at 0.3, 1, or 3 mg/kg i.p.; pilocarpine at 3, 10, or 30 mg/kg i.p.; and arecoline at 3, 10, or 30 mg/kg i.p.) were evaluated.

**Data Analysis.** A Fisher’s exact test was used to compare the frequency of observed side effects. A two-factor ANOVA with re-

---

**Figure 1.** Chemical structures of WAY-132983 ([3R,4R]-(-)-3-(3-hexylsulfonylpyrazin-2-yloxy)-1-azabicyclo[2.2.1]heptane hydrochloride) and xanomeline (3-[4-(hexyloxy)-1,2,5-thiadiazol-3-yl]-1,2,5,6-tetrahydro-1-methyl-pyridine oxalate).
peated measures on one factor followed by a least significant difference test was used to assess differences in core body temperatures.

**Scopolamine-Induced Hyperswimming**

Centrally active muscarinic antagonists significantly increase spontaneous swimming in rats, and muscarinic agonists, such as oxotremorine or arecoline, could reverse the scopolamine-induced increase in swimming distance (Symons et al., 1986).

**Animals.** Male CFW mice (Swiss-Webster derived; Charles River Breeding Laboratories, Wilmington, MA) arrived weighing 25 to 35 g. Animals were housed 10 per cage in a colony room under temperature, light, food, and water conditions as previously indicated.

**Apparatus.** Individual animals were placed in a black Plexiglas, open-top tank (43 cm length × 43 cm width × 15 cm depth) filled with 10 cm of water at room temperature (21–22°C). Four tanks were placed alongside each other to form a square. A video camera, mounted above the tanks, recorded the movement of the mice. The video image was delivered to a San Diego Instruments, Inc. Polytracker video tracking system that was integrated into an HP vectra ES/12 computer.

**Procedure.** Dose-response studies were conducted in mice with scopolamine (0.03, 0.1, 0.3, or 1.0 mg/kg i.p.), a centrally active muscarinic antagonist, or methyl scopolamine (3 or 10 mg/kg i.p.), a peripherally active muscarinic antagonist. Both drugs were administered 30 min before testing. Studies were then conducted with the following muscarinic agonists to determine their ability to attenuate the scopolamine-induced (0.3 mg/kg i.p.) increased swimming distances: oxotremorine (0.1, 0.3, and 1.0 i.p.), pilocarpine (1, 3, 10, or 30 mg/kg i.p.), arecoline (30 mg/kg i.p.), xanomeline (3, 10, or 30 mg/kg i.p.), or WAY-132983 (3, 10, or 30 mg/kg i.p.). In these studies, animals received two injections i.p. 30 min before testing; one control group received vehicle injections, another group received vehicle and then 0.3 mg/kg scopolamine, and the remaining groups received the test compound and then 0.3 mg/kg scopolamine. In the arecoline studies, two different injection schedules were used because of the short duration of action of arecoline (Asthana et al., 1996). One schedule was identical with that used for the other agonists; that is, two injections were administered 30 min before testing; in the second schedule, vehicle or scopolamine was administered as a 30-min pre-treatment and vehicle or arecoline was administered as a 10-min pretreatment.

An individual animal was placed into each tank and allowed to swim freely for 5 min. Total swimming distance (cm) was obtained. A maximum of four tanks were tested at once, and tank assignments for groups were counterbalanced across tests. Individual animal data were discarded when an animal escaped from the tank, when multiple objects were being tracked around a tank (usually due to an escaped animal entering another tank area), or when less than 90% of the testing session was tracked due to animals swimming in shadowed areas, diving under water, or not moving (see figure captions for the frequency of these occurrences). The group sizes noted on the figures refer to animals that were not discarded, and these sizes were sufficiently large for statistical analysis.

**Data Analysis.** The mean swimming distance was analyzed for each group using an ANOVA and the LSD test. A dose of the test compound was considered to be active if the mean was significantly reduced ($P < .05$) from the scopolamine control mean or if it did not differ significantly from the vehicle control mean (because the usual scopolamine difference would have been attenuated).

**RAM Studies (Rats)**

**Animals.** Male CD rats of similar derivation and weight to those used in the previous experiments were housed under similar conditions. After habituation to the facility, animals were individually housed, reduced to 85% of free-feeding weight, and then maintained on a feeding schedule of 10 g/day (Results precision pellets; Bio-Serv, Frenchtown, NJ). Once stable weights were attained, the rats were acclimated to the RAM.

**RAM Procedure.** The maze and the training protocol have been previously reported (Bartolomeo et al., 1997). For the 1-h DNMTS task, four arms were randomly blocked and blocked arm patterns were changed daily. The rat was required to visit all four unblocked arms, retrieving two 45-mg chocolate pellets from each arm (predelay). One hour later, the rat was returned to the maze with all eight arms accessible and was required to retrieve two pellets from those arms that were previously blocked (postdelay). Reentry into an arm was considered an error, including arms previously visited in the predelay. The predelay and the postdelay sessions were limited to 5 min each. Training occurred once per day and continued until animals reached criterion performance ($\leq 3$ postdelay errors (PDEs)) for 3 consecutive days. Different groups of animals were used for the scopolamine and AF64A investigations. Within these groups, animals received various agents in experiments attempting to reverse the increase in PDEs caused by either scopolamine or AF64A. At least 1 week separated dose-response studies of different test compounds to permit a washout period.

**Scopolamine Impairment.** For the scopolamine studies, 0.3 mg/kg (s.c.) scopolamine was administered once to rats in their home cages at least 48 h in advance of the first scopolamine testing session to minimize novel compound effects during RAM testing. During the RAM testing, 0.3 mg/kg (s.c.) scopolamine was administered 30 min before the predelay session. Before initiation of studies with muscarinic agonists, a scopolamine challenge test was conducted to determine which animals would be impaired ($\geq 4$ PDEs) by scopolamine. WAY-123983 (0.1, 0.3, 1, or 3 mg/kg i.p. or p.o.) or xanomeline (0.1, 0.3, 1, 3, or 5.4 mg/kg i.p.) was administered 30 min before the predelay session. Animals received two injections 30 min before the predelay session; one was saline or 0.3 mg/kg scopolamine (s.c.), and the other was saline or test compound (i.p. or p.o.). Latin-square designs were conducted during M1 agonist testing, so each animal was part of each condition during testing. At least 2 days separated each condition. When an animal did not complete the predelay session or made fewer than four choices during the postdelay session, it was determined to have “timed out.” Animals committing two or more timeouts during a Latin-square design experiment were dropped from the data analysis.

**AF64A Impairment.** Previous work with nonselective muscarinic agonists suggested that administration of compounds via miniosmotic pump (s.c.) would attenuate AF64A-induced RAM impairment better than acute administration (Bartolomeo et al., 1997), so WAY-123983 or xanomeline was administered by miniosmotic pumps (s.c.). AF64A was prepared each day of surgery as described by Stewart et al. (1987). Under pentobarbital (50 mg/kg i.p.) anesthesia, AF64A (3 nmol/3 µl/site) or vehicle was infused bilaterally into the lateral ventricles [from bregma: posterior 0.8 mm, lateral ±2.5 mm, ventral (from dura) 4.0 mm, with the stereotaxic incisor bar at 5 mm below the earbar horizontal plane]. Animals recovered for 1 month with food restriction resumed during the fourth week. Animals were then retested in the RAM DNMTS procedure. Baseline criteria of at least 4 PDEs for AF64A-treated animals and 3 or fewer PDEs for vehicle-treated animals were required before drug treatment. WAY-123983 (0.03 mg/kg/day s.c.), xanomeline (0.3 mg/kg/day s.c.), or arecoline (1 mg/kg/day s.c.) was dissolved in 0.2% Tween 80 and administered via an Alzet model 2002 miniosmotic pump. Under halothane anesthesia (2%, with 98% of 19:1 O2/CO2), pumps were inserted s.c. into the dorsal surface of the neck. To minimize peripheral muscarinic effects, 5 mg/kg methyl atropine nitrate i.p. was administered once, immediately after surgery, to the animals receiving WAY-123983 or xanomeline. Animals were tested on days 2, 5, 9, and 14 after implantation. The pumps were removed under halothane anesthesia on day 15. The 4 days of testing during the pump administration were combined, and this mean PDE value was used in statistical analyses. For the WAY-123983 study, the AF64A vehicle data were the combined mean value from a 3-day baseline with...
out pumps. For the xanomeline study, the AF64A/vehicle data were obtained from a single measure during the course of a modified Latin-square design with pumps.

**Choline Acetyltransferase (ChAT) Assay.** Brains were harvested, and the frontal cortex, striatum, and hippocampus were dissected and frozen on dry ice. The animals used in the WAY-132983/AF64A experiment were 17 months of age at harvest, which was 21 weeks after the WAY-132983 RAM study. WAY-132983 was the last drug administered to these animals. The animals involved in the xanomeline/AF64A study were 16 and 18 months of age at harvest, which was 25 weeks after the xanomeline RAM study and 1 day after the last drug administration. For the animals in the WAY-132983 study, only the right hemisphere of brain tissue was used. Procedures for the ChAT activity assay and the protein determination were previously described in Bartolomeo et al. (1997). Briefly, ChAT activity was measured as nmol [3H]acetylcholine formed/mg protein/h.

**Data Analysis.** For both the WAY-132983/scopolamine studies (i.p. and p.o.), a balanced Latin-square design with repeated measures was used on square root transformed data to stabilize vari-ances, followed by a Dunnett’s test. For the xanomeline/scopolamine experiments, a randomized block design was used on square root transformed data, followed by a Dunnett’s test. For the WAY-132983/AF64A data, analysis consisted of a design of two factors with repeated measures on one factor, followed by least significant difference (LSD) comparisons. The xanomeline/AF64A study was analyzed as a one-way layout design with treatment as the factor, followed by pairwise comparisons (LSD).

**DMTS Studies (Primates)**

The animals, which were aged rhesus monkeys and an aged cynomolgus monkey, all had prior training and pharmacological testing. Dose-response curves for WAY-132983 were conducted as well as monitoring of the animals for evidence of side effects.

**Animals.** The study group consisted of six (three males and three females) aged rhesus (Macaca mulatta) monkeys (20–37.5 years old) whose average body weight was 8.2 ± 1.12 kg; and one aged (22 years old) female cynomolgus macaque weighing 2.8 kg. Each animal had previously participated in one or more short-term studies of memory-enhancing agents. Prior drug experience had produced no untoward effects in the animals, and they were allowed at least a 1-month washout period and reestablishment of typical baseline performance before the onset of this study. A drug washout period of 7 days was allowed between each of the three experimental series (dose-response, best dose, and high-dose observational study) of this study. Each animal had performed the DMTS task at least 5 days per week for at least 6 months before the start of the study. Monkeys were individually housed at the Animal Behavior Center of the Medical College of Georgia in stainless steel cages composed of multiple 50 × 28 × 26-inch units. A daily 12-h light/dark cycle was maintained. Toys and foraging tubes were provided routinely, and monkeys were allowed to observe television programs each afternoon after testing to promote psychological well-being. During a test week, monkeys were maintained on a feeding schedule that allowed approximately 15% of their normal daily food intake to be derived from banana-flavored reinforcement pellets awarded for correct responses during testing. Standard laboratory monkey chow, fresh fruits, and vegetables composed the remainder of their daily food intake. Water was available ad libitum.

**Drug Preparation and Administration.** WAY-132983 was stored desiccated at room temperature, dissolved in sterile normal saline just before use, and pipetted onto a sugar cube. Control sessions involved the administration of a placebo (sugar cube). Control (placebo) testing was usually performed on Mondays. The test drug was administered on Tuesdays and Thursdays, with no placebo administered on Wednesdays and Fridays. A test session began 30 min after drug or placebo administration. A minimum washout period of 2 days was maintained between administration of drug doses. A subsequent dose was administered only if a monkey’s performance returned to baseline levels during that period. Performance levels were checked after the placebo administration each week, to establish that the animal’s baseline level of performance had not significantly changed from that of the preceding week. WAY-132983 was used in an ascending dose-response series of the following doses administered p.o.: 0.01, 0.03, and 0.10 mg/kg. After completing this series, an additional dose (0.003 mg/kg) was administered to five of the monkeys (two male rhesus monkeys, monkeys 9 and S11, were not used). An ascending dose series was used to avoid the toxicity to a potential high first dose, which could be encountered by using a randomized dose series.

**DMTS Procedure.** The testing apparatus and the training procedures have been previously described (Terry et al., 1993a). A trial began with the illumination of the colored sample key disk. A key press extinguished the sample light and initiated one of four preprogrammed delay intervals, during which no disks were illuminated. After the delay interval, two choice lights found below the sample key were illuminated. One of the choice lights matched the color of the sample lights. These disks remained illuminated until a monkey pressed one of the two lit keys. Key presses of choice stimuli that matched the color of the sample stimulus were rewarded with a 300-μg banana-flavored pellet. Nonmatching choices were neither rewarded nor punished. Color patterns were fully counterbalanced for side, delay, and match-to-sample. A new trial was initiated 5 s after the second key press on a preceding trial. Monkeys completed 96 trials on each day of testing. The average length of each session for the group was 25 min. Four possible delay intervals between a monkey’s response to the sample light and the presentation of the two choice lights were used: 0-s delay and a short, medium, and long delay. Short, medium, and long delay intervals were individually adjusted to produce stable performance levels approximating the following performance levels: short (75–84% correct), medium (65–74% correct), and long (55–64% correct). The monkeys’ performance for 0-s delay trials averaged 85% correct or greater. For the animals involved in this study, the intervals for zero, short, medium, and long delays were, respectively, 0, 5, 7, and 10 s for one monkey; 0, 5, 7, and 15 s for one monkey; 0, 5, 10, and 15 s for one monkey; 0, 5, 10, and 20 s for two monkeys; 0, 10, 20, and 30 s for one monkey; and 0, 20, 40, and 80 s for one monkey. The rationale for this procedure was to normalize performance based on the varying capabilities of the monkeys.

**Observational Analysis.** At specific time points after drug or placebo administration, the following signs of cholinergic overstimulation were assessed for each monkey: pupillary constriction, ptosis, hypoactivity, reduced food intake, increased water intake, SALIV, abdominal straining, watery stool, soft unformed feces, TREM, retching, and emesis.

**Data Analysis.** Comparisons between the means of several populations were performed using a one- or two-way ANOVA with repeated measures in a paired design, and the differences were considered significant at the P < .05 level. If significant differences in the interactive term (e.g., treatment × dose) were obtained, individual data sets were compared using a paired t test for post hoc analysis. Comparisons for all data were made using raw performance (% correct) scores.

**Drugs**

Oxotremorine sesquifumarate was obtained from Aldrich (Milwaukee, WI). Pilocarpine hydrochloride, scopolamine hydrobromide, methyl atropine nitrate, and arecoline hydrobromide were obtained from Sigma Chemical Co. (St. Louis, MO). Xanomeline and WAY-132983 were obtained from Wyeth Ayerst (Princeton, NJ). AF64A (acetylcholine mustard HCl or ethylcholine aziridinium ion) was obtained from Research Biochemicals Inc. (Natick, MA). Nembutal sodium solution (pentobarbital sodium injection) was obtained from Abbott Laboratories (Chicago, IL); Fluothane (halothane) was obtained from Ayerst Laboratories (Philadelphia, PA).
All compounds were dissolved in 1× Dulbecco’s PBS (without CaCl₂ or MgCl₂·6H₂O; Life Technologies, Grand Island, NY). For the side effect studies, all compounds were administered at a volume of 2 ml/kg b.w.t. for both the i.p. and p.o. routes. For the hyperswimming studies, all compounds were administered at a volume of 10 ml/kg b.w.t. For the RAM studies, all compounds were administered as 1 ml/kg, except AF64A, as noted.

Protocol Approvals

The side effect, hyperswimming, and radial maze procedures were approved by the Wyeth-Ayerst Animal Care and Use Committee. The monkey protocols were approved by the institutional Committee on Animal Use for Research and Education at the Medical College of Georgia.

Results

Generation of Side Effects

Nonselective Cholinergic Agonist Standards. Oxotremorine induced CHROMO, LACRI, and TREM in a dose-dependent fashion (Table 1). The lowest dose, 0.3 mg/kg, did not produce significant levels of these side effects, whereas 1.0 and 3.0 mg/kg did. However, all three doses elicited significant levels of SALIV. All three doses also caused significant hypothermia; 0.3 and 1 mg/kg peaked at 20 min after injection, whereas 3 mg/kg was most hypothermic at the last time point tested, 60 min after injection (Table 1). Three of the 10 animals receiving 3 mg/kg oxotremorine died within 20 min of receiving this dose; these animals were included in the side effect analysis but not in the body temperature assessment.

Pilocarpine dose dependently induced CHROMO, LACRI, and SALIV (Table 1); TREM were not elicited with a dose as high as 30 mg/kg. The lowest dose, 3 mg/kg, did not produce significant side effects, but 10 and 30 mg/kg produced significant side effects. All three doses caused significant hypothermia versus the saline-injected control animals (Table 1). Peak hypothermic effects of 3 and 10 mg/kg occurred at 30 min after injection. At 90 min after injection, the last time point tested, 30 mg/kg exhibited peak hypothermic effects.

Arecoline dose dependently induced LACRI, SALIV, and TREM; CHROMO was not elicited by doses as high as 30 mg/kg (Table 1). SALIV occurred in all rats receiving 10 or 30 mg/kg. The highest dose, 30 mg/kg, also produced significant levels of LACRI and nonsignificant levels of TREM. Each dose of arecoline produced significant hypothermia versus saline-treated control animals. This effect peaked at 10 min after injection, remained significant through 30 min, and returned to saline control levels by 60 min.

WAY-132983 and Xanomeline. WAY-132983 produced a dose-dependent increase in SALIV after either i.p. or p.o. administration. Specifically, the lowest dose of WAY-132983 did not induce SALIV, whereas both 10 and 30 mg/kg elicited significant levels of SALIV after i.p. and p.o. administration (Table 1). WAY-132983 also produced dose-related significant hypothermia after either i.p. or p.o. administration (Table 1). Peak hypothermia was similar by both routes of ad-

### TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Frequency Observed 10 min after Drug</th>
<th>Peak Hypothermia</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHROMO</td>
<td>LACRI</td>
<td>SALIV</td>
<td>TREM</td>
<td>Δ Temperature</td>
</tr>
<tr>
<td>mg/kg i.p.</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td>°C</td>
</tr>
<tr>
<td>Oxotremorine</td>
<td>0.3 (n = 10)</td>
<td>0</td>
<td>10</td>
<td>40*</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>1.0 (n = 10)</td>
<td>90*</td>
<td>90*</td>
<td>90*</td>
<td>80*</td>
</tr>
<tr>
<td></td>
<td>3.0 (n = 10)</td>
<td>50*</td>
<td>70*</td>
<td>70*</td>
<td>80*</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>3.0 (n = 8)</td>
<td>12.5</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10 (n = 8)</td>
<td>87.5*</td>
<td>87.5*</td>
<td>62.5*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (n = 8)</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>0</td>
</tr>
<tr>
<td>Arecoline</td>
<td>3.0 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>100*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (n = 8)</td>
<td>0</td>
<td>62.5*</td>
<td>100*</td>
<td>37.5</td>
</tr>
<tr>
<td>WAY-132983</td>
<td>3.0 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>62.5*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>100*</td>
<td>12.5</td>
</tr>
<tr>
<td>Xanomeline</td>
<td>0.3 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3.0 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10 (n = 7)</td>
<td>0</td>
<td>0</td>
<td>75*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>87.5*</td>
<td>0</td>
</tr>
<tr>
<td>mg/kg p.o.</td>
<td>WAY-132983</td>
<td>3.0 (n = 8)</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>10 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>50*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (n = 8)</td>
<td>12.5</td>
<td>12.5</td>
<td>87.5*</td>
<td>0</td>
</tr>
<tr>
<td>Xanomeline</td>
<td>3.0 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>50*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (n = 8)</td>
<td>0</td>
<td>0</td>
<td>37.5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Last time point tested.
** P < .05 versus vehicle (Fisher’s).
*** P < .05 versus vehicle (LSD).
ministration; 3 mg/kg peaked at 30 min, 10 mg/kg peaked at 60 min, and 30 mg/kg peaked at 120 min after injection. Xanomeline also produced a dose-dependent increase in SALIV. Specifically, the lowest doses (0.3 and 3.0 mg/kg) of xanomeline did not induce SALIV, whereas both 10 and 30 mg/kg i.p. elicited significant levels of SALIV (Table 1). Oral administration of xanomeline yielded a significantly high incidence of SALIV at 10 mg/kg only (Table 1). Xanomeline produced significant hypothermic effects at 30 mg/kg i.p., which lasted 90 min, versus saline-treated control animals (Table 1). Doses of 0.3 and 3 mg/kg did not affect core body temperature. In a separate experiment (with a saline-treated control group), 10 mg/kg xanomeline i.p. caused a smaller, but significant, decrease in body temperature that lasted through 30 min (Table 1). Oral administration of xanomeline did not yield significant changes in body temperature at 3, 10, or 30 mg/kg (Table 1).

Scopolamine-Induced Hyperswimming

Cholinergic Antagonism. Scopolamine produced a dose-dependent increase in swimming distance with a minimum effective dose of 0.3 mg/kg i.p. (Fig. 2A); this dose was used to generate scopolamine-induced hyperswimming in subsequent experiments. Methyl scopolamine, at doses as high as 10 mg/kg i.p., was unable to cause a significant increase in swimming distance (Fig. 2A).

Nonselective Cholinergic Agonist Standards. Oxotremorine produced a dose-dependent attenuation of scopolamine-induced hyperswimming. Although oxotremorine significantly lowered the scopolamine-induced hyperswimming at 0.1 mg/kg i.p., at this dose the mean swimming distance remained significantly greater than that of the saline-treated control animals (Fig. 2B). The doses of 0.3 or 1 mg/kg oxotremorine i.p. significantly decreased the scopolamine-induced hyperswimming whereas not producing effects signific-

---

**Fig. 2.** Induction of hyperswimming by cholinergic antagonist administration and attenuation by known cholinergic agonists. A, four doses of scopolamine (i.p) and two doses of the peripherally active methyl scopolamine i.p. were administered to mice 30 min before placement in the swim tanks. During the methyl scopolamine testing, three animals escaped and nine animals were not included due to insufficient tracking. B, three doses of oxotremorine i.p. were administered with scopolamine 30 min before placement in the swim tanks. C, four doses of pilocarpine i.p. were administered with scopolamine 30 min before placement in the swim tanks. During the pilocarpine testing, three animals escaped and six animals were not included due to insufficient tracking. D, arecoline (30 mg/kg i.p.) was either administered with scopolamine 30 min before placement in the swim tanks or 20 min after scopolamine at 10 min before placement in the swim tanks. During the arecoline testing, three animals escaped, two animals were not used due to multiple objects being tracked, and nine were not included due to insufficient tracking.
Pilocarpine produced a dose-dependent attenuation of scopolamine-induced hyperswimming. Specifically, pilocarpine significantly lowered the scopolamine-induced hyperswimming at 30 mg/kg i.p., whereas at 10 mg/kg i.p., the effects of pilocarpine were not significantly different from the saline- or the scopolamine-treated control animals (Fig. 2C).

Arecoline (30 mg/kg) did not significantly reduce scopolamine-induced hyperswimming regardless of injection schedule (Fig. 2D). When 30 mg/kg arecoline i.p. was administered with scopolamine, the mean swimming distance did not change appreciably. When 30 mg/kg arecoline i.p. was administered 20 min after the scopolamine injection, the mean swimming distance was lower but remained significantly higher than that of the saline-treated control animals. Four of the seven saline-treated mice received the second saline injection 20 min after the first one. There were no significant differences in mean swimming distance between these two conditions, so these groups were combined for the purposes of the repeated measures comparison with the other groups and the combined mean value is presented on Fig. 2D.

WAY-132983 and Xanomeline. WAY-132983 and xanomeline both dose dependently attenuated scopolamine-induced hyperswimming. WAY-132983 at all doses tested (3, 10, or 30 mg/kg i.p.) significantly reduced scopolamine-induced hyperswimming (Fig. 3A). Xanomeline produced significant reduction of scopolamine-induced hyperswimming at the lower doses of 3 or 10 mg/kg i.p., but swimming at these doses remained significantly greater than that of the saline-treated group (Fig. 3B). At 30 mg/kg i.p., xanomeline yielded both significant attenuation of scopolamine-induced hyperswimming and nonsignificant differences with the saline-treated control animals.

RAM Studies (Rats)

Scopolamine Impairment. After i.p. administration, WAY-132983 completely restored scopolamine-impaired performance to baseline levels. WAY-132983 lowered PDEs compared with scopolamine-treated animals at all doses tested (0.1, 0.3, 1, or 3 mg/kg i.p.), and 0.3 and 3 mg/kg significantly reduced scopolamine-induced increased PDEs (Fig. 4A). Also, for all of the doses of WAY-132983 tested, PDEs were not significantly different from those of the saline control condition. When administered p.o., WAY-132983 attenuated the increase in PDEs produced by scopolamine. Specifically, p.o. administration of 0.1, 0.3, 1, or 3 mg/kg WAY-132983 resulted in lower PDEs compared with scopolamine/vehicle and no significant differences versus saline/saline treatment (Fig. 4B).

Two separate (i.e., with different animals in each) dose-ranging studies using xanomeline were conducted, and 0.3 mg/kg scopolamine s.c. induced significantly increased PDEs in both studies (Fig. 4, C and D). In the low-range design, xanomeline attenuated the increase in PDEs produced by
scopolamine but did not completely restore performance. Specifically, animals treated with 0.1, 0.3, or 1 mg/kg xanomeline i.p. did not commit significantly more PDEs than saline-treated control animals, nor did these doses significantly lower PDEs compared with the scopolamine/saline condition (Fig. 4C). When the dose range was increased for xanomeline, 1 and 3 mg/kg i.p. doses restored performance to baseline levels. Xanomeline significantly lowered PDEs versus the scopolamine/saline-treated animals, without significant differences from the saline/saline treatment PDEs (Fig. 4D). The highest dose tested, 5.4 mg/kg i.p., of xanomeline produced significantly more PDEs compared with the saline/saline control animals.

AF64A Impairment. WAY-132983 (0.03 mg/kg/day s.c.), xanomeline (0.3 mg/kg/day s.c.), or arecoline (1 mg/kg/day s.c.; Fig. 5) administered to AF64A-treated animals produced significantly fewer PDEs than those observed in AF64A/vehicle-treated animals but did not restore responding to baseline levels; that is, the animals treated with the indicated doses of WAY-132983, xanomeline, or arecoline had significantly higher PDEs than the paired vehicle/vehicle animals.

ChAT Assay. ChAT activity was significantly reduced in hippocampi from AF64A-treated animals from both the WAY-132983 and the xanomeline study (Table 2). Striatal and frontal cortical tissue did not display any significant changes in ChAT activity due to AF64A.

DMTS Studies ( Primates)

Oral Administration of Placebo. The seven animals in the study group approximated DMTS baseline performance according to the criteria described above (see Materials and Methods), and they exhibited a significant delay-dependent decline in performance ($F_{3,18} = 102.0$, $P < .001$). Performance for the group after the administration of placebo ($n = 19$) averaged $93.5 \pm 1.13$, $73.3 \pm 2.55$, $69.1 \pm 1.31$, and $58.1 \pm 1.74$% correct, respectively, for zero, short, medium, and long delay intervals. The mean for each group was significantly different from that of every other group ($P < .05$).

Most Effective Dose and Delay Intervals for Oral Administration of WAY-132983. Of the seven monkeys in the study group, only one monkey (monkey 9) failed to respond to any dose of WAY-132983. Monkey 9 was a male rhesus, and the oldest (37.5 years) and largest (12.2 kg) of the group. Before this study, monkey 9 responded to other drugs administered to enhance task performance, such as nicotine. Although it was possible that monkey 9 might respond to other doses of WAY-132983, for the purposes of this study, he...
was removed from statistical consideration. The removal of this monkey had no affect on the overall statistical significance of the data.

WAY-132983 significantly improved DMTS performance for both short and long delay intervals (Fig. 6). For short delay intervals, significant improvement amounting to about 27% over baseline performance was obtained at the 0.03 mg/kg dose. In addition, there was a dose-dependent improvement in DMTS performance as measured 24 h after drug administration. In this case, the two highest doses produced significant performance increases relative to baseline. For long delay intervals, significant improvement amounting to 20 to 25% over baseline performance was obtained at the 0.01 and 0.1 mg/kg doses. As with the short delay performance, 24 h after drug administration, significant improvement in task performance was still evident for long delay at the 0.1 mg/kg dose. There was no significant improvement in task performance for either zero or medium delay intervals.

A "best dose" (dose that improved task performance to the greatest extent across all delay intervals) was selected for each monkey (responder) from the dose-response data. Three of the animals’ best dose was 0.10 mg/kg, two monkeys’ best dose was 0.03 mg/kg, and one monkey’s (the cynomolgus) best dose was 0.01 mg/kg (average best dose for the group was 0.062 ± 0.0174 mg/kg). The results of the Best Dose analysis are presented in Fig. 7. On the day of drug administration, overall (across all delays) DMTS performance was significantly improved compared with placebo levels of performance. This overall improvement generally reflected improved performance for both short and long delay intervals. Although there was some improvement for zero and medium delay intervals, these increases were not statistically significant. On the day after the administration of WAY-132983, performance improvement continued to be maintained overall, particularly for short and long delay intervals. The level of enhancement 24 h after drug administration was similar to that measured on the same day (Fig. 7).

Observational Study of Highest Dose of WAY-132983. Sugar cubes (drug or placebo) were administered to seven monkeys at 9:10 AM according to the paradigm described in Materials and Methods. The monkeys were observed for clinical signs of cholinergic overstimulation at 15, 30, and 60 min and 2, 4, and 6 h after the administration of 0.10 mg/kg WAY-132983 or placebo. No signs of cholinergic overstimulation were recorded for any of the monkeys at any of the time checks. When examined on the morning of the day after the first administration, no signs were recorded in any animal;
therefore, the second set of sugar cubes was given. No signs were recorded at the 15-min check for any of the animals. At the 30-min check, blood was visible on the perch in one animal’s (monkey LB, the cynomolgus) cage. The code for this animal was broken, and it was determined that she had been administered drug on the first day. The bleeding was moderate and appeared to stop within 20 min. At the 4-h check point, there was evidence of loose stool and fresh blood at the bottom of the cage. At the 6-h check point, the monkey appeared depressed and hunched over in the cage. Despite some loss of appetite, DMTS performance was normal the next day and reward pellets were consumed. After a routine test for tuberculosis the week after testing, which required sedation, the animal’s health continued to deteriorate and she died within 2 weeks. A preliminary autopsy report indicated that the liver and mesenteric vessels were engorged with blood. There was an intussusception distal to the cecocolic junction, and an area of hemorrhage was cranial to the intussusception. Other organs were unremarkable.

It also should be pointed out that monkey LB exhibited drooling 2 h after receiving the 0.1 mg/kg dose during the initial DMTS study and was the only animal to do so. The animal was a clear responder in terms of DMTS task improvement to WAY-132983, but she stood out from the other animals in that her best dose was the lowest (0.01 mg/kg). LB was also the smallest animal of the group, weighing only 2.8 kg. None of the other six monkeys ever exhibited these signs, nor did they exhibit any signs of cholinergic overstimulation.

**Discussion**

These experiments demonstrate that WAY-132983 produces effects in vivo consistent with its profile as an M1 preferring agonist. Beneficial cognitive effects in tests of cho-
linergic hypofunction induced by scopolamine or AF64A in rodents and aging in primates were evident. The absence of limiting side effects at doses producing cognitive benefits supports the potential clinical use of WAY-132983 in AD.

Generation of Side Effects

Nonselective Cholinergic Agonists. Oxotremorine was the most potent muscarinic agonist to induce side effects, producing CHROMO, LACRI, SALIV, and TREM at 1 mg/kg, which is consistent with other reports (Witkin et al., 1987; Clement, 1994). Pilocarpine caused CHROMO, LACRI, and SALIV without evidence of TREM, which is consistent with Clement (1994) but differs from Witkin et al. (1987), who reported TREM at 200 mg/kg, which was the lowest dose tested. Arecoline readily produced SALIV, and the highest dose tested induced LACRI without CHROMO or TREM. Our arecoline results also differed from those of Witkin et al. (1987). When they tested arecoline, the lowest dose (100 mg/kg) produced LACRI in 16.7% of treated rats, whereas we obtained a higher incidence with 30 mg/kg.

Cholinergic agonists induce hypothermia (Sen and Bhat-}

tacharya, 1991; Shannon et al., 1994), and our results are
consistent with this, because doses that did not elicit other
side effects produced significant hypothermia, suggesting a
very sensitive measure. As expected because of its short
half-life, the hypothermic effects of arecoline were not dose-
related and were transient for all doses, peaking 10 min after
injection and returning to baseline by 60 min.

WAY-132983 and Xanomeline. WAY-132983 and xa-
nomeline were equipotent at inducing cholinergic side ef-
facts. Both drugs caused SALIV. When administered p.o.,
each compound retained its potency for eliciting SALIV. Both
pharmacological (Schiavone and Brambilla, 1991; Sanchez
and Løve Lembøl, 1994) and molecular (Dai et al., 1991)
research suggests that SALIV is predominantly an M3-me-

Fig. 7. Effect of administration of WAY-132983 on DMTS performance by aged monkeys. Data are presented as the best dose. Each value represents the mean ± S.E. Overall mean scores were significantly different from control levels of performance (F2,10 = 14.5, P < .001). Short delay means were significantly different from control levels of performance (F2,10 = 30.7, P < .001). Long delay means were significantly different from control levels of performance (F2,10 = 28.6, P = .008). *Significantly different from placebo baseline performance level (P < .05, post hoc test).

Scopolamine-Induced Hyperswimming

Scopolamine, but not methscopolamine, significantly in-
creased swimming distance, which is consistent with a pre-
vious study demonstrating that central, but not peripheral,
acting cholinergic antagonists increase swimming distance
(Symons et al., 1986). Oxotremorine and pilocarpine, but not
arecoline, attenuated scopolamine-induced increased swim-
m
ing distance. Oxotremorine and arecoline were previously
reported to reverse the scopolamine-induced increased swim-
m
ing distance (Symons et al., 1986). Although that report did
not mention effective doses, administration times, or routes,
our results with arecoline might reflect a below-range dose,
or because arecoline is short-lived (Asthana et al., 1996),
perhaps there was an effective time window not coincident
with the assessed time points. Both WAY-132983 and xa-
nomeline reduced scopolamine-induced increased swimming
distance consistent with their muscarinic agonist profiles.

RAM Studies (Rats)

Scopolamine Impairment. Scopolamine consistently in-
creased PDEs, which is in agreement with the literature
(Pilcher et al., 1997). WAY-132983 (i.p. and p.o.) reduced
scopolamine-induced increased RAM PDEs at all doses. In-
vestigation of a larger dose range would provide the mini-
m
mum effective dose for WAY-132983.

Xanomeline also reduced PDEs versus scopolamine-in-
duced increased RAM PDEs, but the highest dose tested (5.4
mg/kg) was ineffective. This pattern of cognitive efficacy,
perhaps suggesting an inverted U-shaped relationship, has
been demonstrated with other cholinergic compounds (areco-
line: Soncrant et al., 1993; Bartolomeo et al., 1997; hupera-
zine A: Xiong et al., 1995).
**AF64A Impairment.** AF64A treatment increased PDEs, which is consistent with previous findings (Bartolomeo et al., 1997). WAY-132983, xanomeline, and arecoline reduced PDEs. Dose-response curves for xanomeline and WAY-132983 would define the optimal dose for decreasing the AF64A-induced RAM impairment. Animals treated with xanomeline, WAY-132983, or arecoline made more PDEs than vehicle/vehicle control animals, allowing the possibility that a dose-response assessment might reveal doses at which no difference from vehicle/vehicle control animals would be seen.

The ChAT assay verified that AF64A compromised hippocampal presynaptic cholinergic terminals, whereas other areas (frontal cortex, striatum) were unchanged. Two colonies of animals the same age (16–18 months) and with similar postAF64A intervals to ChAT activity assessment (13–15 months), were used for the AF64A impairment studies. In both colonies the reduction in hippocampal ChAT was 20% or greater, but differences in absolute values of ChAT were seen. These differences may be attributed to variability in running separate ChAT assays for each colony.

**DMTS Studies (Primates)**

WAY-132983 produced improvement in DMTS performance after p.o. administration to aged monkeys that were well trained in the task. This improvement occurred primarily for trials associated with both short and long delay intervals. This bimodal temporal benefit may reflect two distinct mnemonic properties of WAY-132983: 1) improvement in working memory reflected in the positive effects at long delay intervals and 2) reduction of distractibility (increased attention) improving short delay performance. Short delay-associated performance is more sensitive to stimulus-relevant distractors than are medium and long delay performances (Prendergast et al., 1998). Task-relevant distractors may be less effective in disrupting longer duration delay interval trials, possibly because they allow more time for the animal to consider what the correct stimulus was. In the present experiment, the medium delay interval performance was not improved to the extent observed for short and long intervals. It is possible that enhanced attention might increase proactive interference affecting primarily medium delay trials. Thus, medium delay trials may have been of sufficient length to be impaired by proactive interference but too short to benefit from improved working memory.

The only nonresponder of the group was the oldest and largest monkey. His size and/or age could explain his lack of responsiveness, possibly indicating responsiveness in some other (possibly higher) dose range. Another monkey, a cynomolgus macaque, appeared to exhibit a toxic and possibly fatal reaction to WAY-132983. However, it cannot be determined whether the monkey had a preexisting condition or whether her sensitivity to the drug was species-specific. One other potential variable is the animal’s body weight, which was the lowest of the group. The death of the cynomolgus monkey in the study requires further investigation. The six rhesus monkeys treated with WAY-132983 not only survived but also exhibited no obvious side effects, suggesting that perhaps a strain difference contributed to the death of the cynomolgus monkey.

The rodent and primate experiments agree, indicating that working memory is improved by WAY-132983. Previous work in primates has indicated that other cholinergic modulators, such as acetylcholinesterase inhibitors (physostigmine: Terry et al., 1993b; velnacrine: Jackson et al., 1995) or nicotinic agonists (ABT-418: Buccafusco et al., 1995), can also improve DMTS performance. Differences in profile and magnitude of response between WAY-132983 and other cholinergic modulators may be important. For example, WAY-132983 affected both short and long delay intervals, but other drugs have tended to improve long delay intervals only (e.g., velnacrine: Jackson et al., 1995; ABT-418: Buccafusco et al., 1995). Physostigmine affected short delays only on the day of drug administration and long delays only 24 h later (Terry et al., 1993b). In combination with clonidine, physostigmine improved both medium and long delay intervals on both drug administration day and 24 h later. The velnacrine effects also persisted 24 h, similar to the present results for WAY-132983, but ABT-418 did not show this persistence. This persistence of effect may suggest the presence of a long-lived active metabolite or the initiation of a long-term neural change (such as long-term potentiation) underlying the improved performance. The magnitude of effects of the other cholinergic modulators varied within 8 to 16%, whereas the effects after WAY-132983 were 15 to 25% above baseline. These data suggest that WAY-132983 would be beneficial in treating patients with AD and that those effects might be qualitatively better (two mnemonic effects) in that they might be more persistent and larger in magnitude than those seen with either acetylcholinesterase inhibitors or nicotinic agonists.

**Conclusions**

WAY-132983 exhibits a pharmacological profile consistent with its neurochemical profile as an M1 preferring agonist (Tasse et al., 1997) and similar to that of xanomeline, a previously described M1 preferring agonist (Shannon et al., 1994). WAY-132983 demonstrates p.o. availability supported by hypothermic effects, attenuation of scopolamine-induced RAM impairments, and improvements in a visual DMTS task in aged monkeys. Of six rhesus monkeys tested in the DMTS, none displayed any cholinergic overstimulation, indicating favorable separation between doses of WAY-132983 producing cognitive benefit and those producing unwanted effects. The rodent data also support this, with at least a 10-fold difference between doses producing SALIV and doses producing cognitive improvement in impaired RAM tasks.

Although the cognitive tests reported in this study do not model AD pathology, WAY-132983 attenuated impairments due to cholinergic dysfunction, which is associated with AD (DeKosky et al., 1992). Future studies should determine the effectiveness of WAY-132983 to reduce the AD-associated pathologies of amyloid plaques and neurofibrillary tangles, because muscarinic agonists increase the formation of a soluble, nonaggregating amyloid fragment rather than the insoluble, aggregating β-amyloid protein fragment (Eckols et al., 1995). The effects on amyloid precursor protein processing are mediated by protein kinase C stimulation (Slack et al., 1993) resulting from M1 receptor activation. Such an effect would be favorable in patients with AD in combination with the cognitive benefits expected based on the present animal experiments.
Acknowledgments

We thank Cathy Hoffman and Geoff Mihalenko for their assistance in stereotactic surgeries and brain harvesting, Mike Weaver for his insights and construction of the swim tank assembly, Ed Godfrey for working on the video camera arm apparatus, Charlie Vorhees for adding orbital lighting into the swim tank room, Mike Weaver and Charlie Vorhees for their efforts in building and maintaining the radial maze, Bob LaForge for his work in generating the computer program and the electronic interfaces that automated the maze, Dr. Youping Huang for the statistical analyses, and Erica DiPaola for her work in preparing the manuscript.

References


Send reprint requests to: Dr. Carl Boast, CNS Disorders Division, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000. E-mail: boast@war-wyeth.com