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Research Article

Ranitidine Analog, JWS-USC-75IX, Enhances Memory-Related Task Performance in Rats

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The purpose of this study was to evaluate potential cognitive effects and nicotinic receptor binding affinity of a ranitidine analog previously found to antagonize M_2 muscarinic-cholinergic receptors and acetylcholinesterase (AChE) in vitro. Several doses of JWS-USC-75IX (JWS) were evaluated in rats in three memory-related tasks, a passive avoidance (PA) paradigm, the Morris Water Maze (MWM), and a delayed stimulus discrimination task (DSDT). Rat groups (n per dose) were as follows: PA = Wistar, n = 20–25; MWM = 1-year-old Long-Evans, n = 7-8; and DSDT, Wistar, n = 6. In PA, JWS (1.0 mg/kg) improved scopolamine (SCOP)-induced deficits in learning to avoid an unsafe region of a shuttle box as indicated by both latency and learning frequency analysis. In the MWM, JWS (0.1, 0.5, and 1.0 mg/kg) improved "spatial navigation learning" as demonstrated by the improved ability of rats to locate a hidden platform on day 2 of an 8-day training schedule. Two doses (0.1 and 0.5 mg/kg) also improved "spatial bias" for the previous platform location when tested on day 9. JWS was not particularly effective at reducing SCOP-induced deficits in the MWM and failed to improve DSDT accuracy in a dose-dependent manner across delays. However, repeated optimal doses significantly improved DSDT accuracy at medium and long, presumably more difficult, delay intervals. JWS did not demonstrate a high affinity at nicotinic receptors as indicated in [^{3}H]-cytisine displacement assays. The data thus indicate moderate improvements in the performance of three memory-related tasks in both young and middle-aged rodents of two strains administered JWS. These results appear to substantiate the hypothesis that antagonizing both M_2 cholinergic receptors and AChE offers a potential means of improving cognition and supports the potential use of this agent (or similar compounds) in disorders of memory. Drug Dev. Res. 47:97–106, 1999.

Key words: memory; cholinergic; rat; M_2 receptor; acetylcholinesterase; histamine

INTRODUCTION

The important role of the septohippocampal cholinergic system in a variety of mnemonic processes and overall cognitive function (and dysfunction) is well documented [for review, see Palmer, 1996]. Degeneration of these pathways and reductions in several cholinergic markers are among the most salient and reproducible neurochemical findings in postmortem Alzheimer's disease (AD) brains [Coyle et al., 1983; Perry, 1986]. Moreover, it has come to light in recent years that cholinergic neurons influence the regulation of β-amyloid precursor processing and abnormalities in these neurons may lead to β-amyloid deposition and formation of toxic neuritic plaques [Kosik, 1992; Selkoe, 1993]. Taken together, the accumulated evidence substantiates the hypothesis that

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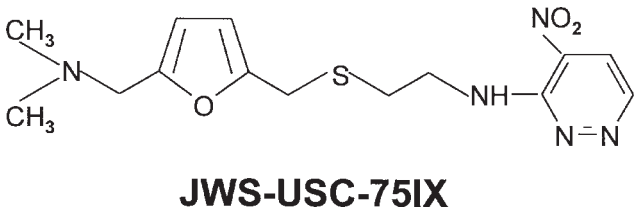
compounds which enhance central cholinergic neurotransmission should be of potential therapeutic value for conditions such as AD and Huntington's disease [Spokes, 1981] where cholinergic hypofunction is consistently reported. In the case of AD, the agents utilized thus far to exploit the "cholinergic hypothesis" have been hindered clinically by peripheral side effects, lack of selectivity, and poor reliability, and have thus been generally disappointing from a therapeutic standpoint. Nevertheless, the acetylcholinesterase inhibitor (AChEI) tacrine has demonstrated clear (albeit modest) benefits to some patients, and thus offers one method of treating a previously untreatable disease [Schneider, 1993, 1994]. Recently developed agents of this class with reduced systemic side effects (e.g., donepizil-Aricept®) may further enhance their therapeutic potential [Schwartz et al., 1991; Rogers and Friedhoff, 1994]. An additional limitation to this category of agents and direct agonists at muscarinic receptors, however, is their interaction with presynaptic inhibitory M_2 autoreceptors, which may produce feedback inhibition [Mash et al., 1985]. This action likely inhibits acetylcholine release and may contribute to the poor reliability of these compounds. It has been suggested that activation of M_2 autoreceptors by endogenous acetylcholine may even exacerbate the cholinergic deficiency observed in AD [Mash et al., 1985; Egan and North, 1986].

In relatively recent years, several muscarinic-cholinergic agonists and antagonists have been developed with sufficient receptor subtype selectivity to possibly address this issue. For example, selective M_2 receptor antagonists such as BIBN-99 [Doods et al., 1993, Quirion et al., 1995] and AF-DX 116, [Lapchak et al., 1989; Packard et al., 1990], have been reported to increase synaptic acetylcholine levels and improve the performance of memory tasks in rodents. Several oxotremorine analogs [e.g., BM-5; Engstrom et al., 1987] and pilocarpine isosteres [e.g., thiopilocarpine; Shapiro et al., 1992] exhibit M_1 agonist activity as well as M_2 antagonist properties and are of potential value.

A few years ago, we synthesized and evaluated biochemically a series of 26 analogs of ranitidine for the purpose of creating nontoxic AChEIs. Several of these compounds were found to possess potent AChEI properties and low toxicity profiles [Valli et al., 1992]. Of this series, one compound, JWS-USC-75IX (JWS; see Fig. 1), was found to possess AChEI activity in vitro (IC_{50} ~0.47 μM), as well as potent M_2 receptor antagonist activity in mouse cerebral cortex (IC_{50} ~60 nM). It had been hypothesized previously that in the therapy of AD, an M_2-selective antagonist might be very useful given in conjunction with an AChEI to prevent the acetylcholine from decreasing its own release [Quirion, 1993; Mash et al., 1985]. This compound combined both features in a single agent and offered a significant potential for improved reliability over presently available compounds for the treatment of hypocholinergic diseases. Therefore, the purpose of this study was to test the hypothesis that enhancement of learning and memory in rodents could be achieved by combining M_2 receptor antagonism with AChEI using JWS. Based on certain structural features of the compound (namely, the dimethylamino methyl moiety), we also were interested in the possibility that JWS might bind nicotinic-cholinergic receptors.



JWS-USC-75IX

Fig. 1. Chemical structure of JWS-USC-75IX.

MATERIALS AND METHODS

Animals

100 Male Wistar rats (45 days old) and 75 1-year-old male Long-Evans (hooded) rats (retired breeders) were obtained from Harlan Sprague-Dawley, Inc., and housed individually in stainless steel mesh cages in a temperature controlled room (25°C) with a 12-h light/dark cycle. Upon arrival, each animal was provided with water and standard rodent chow (NIH-07 formula) ad libitum. All procedures outlined in this report were approved by the Medical College of Georgia, IACUC.

Chemicals

JWS-USC-75IX (3-[[[2-[[(5-dimethylaminomethyl)-2-furanyl]methyl]thio]ethyl]amino]-4-nitropyridazine) was synthesized as described previously [Valli et al., 1992].

Passive (Inhibitory) Avoidance

Wistar rats (20–25 per group) were used for all passive avoidance experiments.

Procedure

Passive avoidance training was conducted in a dimly lit room with a shuttle cage (Coulbourn Instruments, Lehigh Valley, PA) having a retractable (guillotine) door dividing the cage into two equal compartments. Rats were placed in this room each day, 30 min before the beginning of the experiment, then administered one of the following drug combinations IP at 30 and 15 min, respectively, before testing: saline + saline; scopolamine 0.5 mg/kg + saline; scopolamine 0.5 mg/kg + JWS 0.5 or 1.0 mg/kg. A training trial was initiated by placing a rat into the left (safe) compartment and fastening the top of the apparatus to prevent escape. Approximately 20 sec later a bright light

came on in this side of the chamber and the guillotine door raised, allowing entry into the dark (unsafe) compartment. When the rat completely crossed into the dark compartment, the guillotine door immediately lowered and an inescapable, 0.8 mA (scrambled) foot shock was delivered through the grid floor for 5 sec. The rat was then removed from the apparatus and returned to its home cage. Forty-eight hours later a retention trial was performed in a similar manner as the training trial, except that no injections were given and no shock was delivered if the rat crossed into the unsafe compartment. The latency to enter the dark chamber (step-through latency) and learning frequencies (number of rats not crossing into the unsafe side) during the retention trial were used as measures of memory for the training experience. Rats which did not cross over into the dark chamber after 300 sec were given a latency score of 300 sec and designated as having learned the task (for frequency analysis).

Water Maze Experiments

Water maze experiments were conducted in 1-year-old Long Evans rats to determine the effect of JWS across a range of doses (compared to saline controls) on latencies to locate a hidden platform and to determine if JWS had the potential to reverse scopolamine-induced deficits in learning.

Testing apparatus

Maze testing was performed in a circular pool (diameter 180 cm, height 76 cm) made of plastic (Bonar Plastics, Noonan, GA) with the inner surface painted black. The pool was filled to a depth of 35 cm of water (maintained at 25 ± 1°C) which covered an invisible (black) 10-cm square platform. The platform was submerged approximately 1 cm below the surface of the water and placed in the center of the northeast quadrant. The pool was located in a large room with a number of extra-maze visual cues, including light-reflective geometric images (squares, triangles, circles, etc.) hung on the wall, diffuse lighting, and black curtains used to hide the experimenter and the awaiting rats. Swimming activity of each rat was monitored via a ccTV camera mounted overhead, which relayed information including latency to find the platform, total distance traveled, time and distance spent in each quadrant, etc., to a video tracking system (Poly-Track, San Diego Instruments, San Diego, CA). Tracking was accomplished via a white rat (or white portion of rat, in the case of Long Evans rats) on a black background.

Procedures

Hidden platform test. Each rat was given four trials per day for 8 consecutive days. On days 1–8, a trial was initiated by placing the rat in the water facing the pool wall in one of the four quadrants (designated NE, NW, SE, SW). The daily order of entry into individual quadrants was randomized such that all four quadrants were used once every day. For each trial, the rat was allowed to swim a maximum of 90 sec in order to find the hidden platform. When successful, the rat was allowed a 30-sec rest period on the platform. If unsuccessful within the allotted time period, the rat was given a score of 90 sec and then physically placed on the platform and also allowed the 30-sec rest period. In either case, the rat was immediately given the next trial (ITI = 30 sec) after the rest period. Daily swim speeds were recorded by dividing the distance traveled during location of the platform (in cm) by the latency (in seconds).

Probe trial (transfer test). On day 9, two trials were given (and averaged) in which the platform was removed from the pool to measure "spatial bias" [Morris, 1984]. This was accomplished by measuring the time and distance traveled in each of the four quadrants.

Visual platform test. Immediately following the probe trial, the platform was reintroduced to the pool in the quadrant opposite the original position (SW quadrant) with a highly visible (light-reflective) cover attached to the platform which was raised above the surface of the water (approximately 1.5 cm). The curtains were drawn and lighting was changed such that only the visible platform was illuminated and the extra-maze cues were no longer visible. Each rat was given one trial in order to acclimate to the new set of conditions and locate the platform visually. This was accomplished by lowering the rat into the water in the NE quadrant and allowing location of the platform. No time limit was placed on this first trial. The rat was then immediately given a second trial in the same manner and the latency to find the platform measured as a comparison of visual acuity.

Drug administration

The rats were placed in groups of 7–8 and administered one of the following drug combinations IP at 30 and 15 min, respectively, before testing (saline = sal, JWS USC 75IX = JWS, scopolamine = SCOP): sal-sal (1.0 ml/kg), sal-JWS 0.1 mg/kg; sal-JWS 0.5 mg/kg; sal-JWS 1.0 mg/kg: SCOP 0.5 mg/kg-sal; SCOP-JWS 0.1 mg/kg; SCOP-JWS 0.5 mg/kg; SCOP-JWS 1.0 mg/kg

Delayed Stimulus Discrimination Task (DSDT)

One week after arrival, six male Wistar rats were restricted to a daily feeding of 18 grams per day (approximately 80% of their ad libitum consumption). Additional food was given on weekends and holidays to maintain the weight of each rat at approximately its freely fed weight.

Behavioral testing: DSDT

Apparatus. Rats were trained and tested as described previously [Terry et al., 1996, 1997] in operant

chambers enclosed in ventilated, sound- and light-attenuated cubicles. Dim chamber illumination was provided by a small 3-watt house light located on the cubicle ceiling. Each chamber was fully computer automated with levers centered on each side of a feedbox. Each lever, one on the right and one on the left, could be depressed to earn food pellet rewards. The designation of which lever was associated with a food reinforcer was determined by the presentation of either a light or a tone. The animals were trained to discriminate between the light and the tone, i.e., the reward was provided only on the right side following a trial which began with the presentation of a light, whereas following trials beginning with the presentation of a tone, only a response to the left side was rewarded. The duration of each stimulus was 3 sec. Immediately following the stimulus, variable delay intervals (1 of 3), each associated an equal number of times with the light and the tone, were presented repetitively to comprise a daily test session of 64 trials. During delay intervals, the rats were sequestered behind retractable doors which remained closed to prevent access to the levers. At the end of the delay, the doors quickly opened, allowing access for lever selection by the rat. The doors remained open for 5 sec to allow time for the rat to choose a lever and if a correct choice was made, consume its reward. Finally, the doors were gently closed for a total intertrial interval of 10 sec. If an incorrect choice was made, no reward was given and the next trial was initiated.

Training procedure. The rats were trained 5 days per week, Monday through Friday. Beginning 2 weeks after arrival, they were trained to lever press (without a presented stimulus) on a 30-trial continuous reinforcement schedule (45 mg pellets, Bioserv, Frenchtown, NJ) in which the doors were always open and either lever press was rewarded. Once a particular rat was routinely successful at obtaining 30 pellets, it was graduated to a program in which 32 each of the light and tone configurations were randomly distributed throughout 64 trials by means of a Gellerman series [Gellerman, 1933]. The doors closed immediately before the stimulus presentation and remained closed throughout a 1-sec delay, and only the correct lever selection was rewarded. The delays were increased to 3, then to 5 sec when accuracy approached 90%. Once the animals learned to discriminate correctly during trials involving 5-sec delays (criterion = ≥70% accuracy), two additional delays were employed and randomly distributed with the 5-sec delays throughout the session for a total of 64 trials. The three delays were designated as short, medium, and long, and were adjusted empirically (with the exception of short delays, which were always 5 sec) and varied according to the ability of each individual rat to perform the task. The general criteria for length of delay included an accuracy of ~75–85% correct at short delays, 65–75% correct at medium delays, and 55–65% at long delays.

Drug Administration

Saline. When the rats were able to maintain DSDT accuracy above chance at long delay intervals of at least 20 sec with stable baselines (defined as accuracy within the range defined above for each delay for a period of at least 10 consecutive sessions), IP saline injections (1.0 ml/kg) were initiated. Daily saline administration continued until baseline performance was unaffected by injection (typically 3–5 sessions). Thereafter, saline administration continued throughout the course of the study on nondrug days.

JWS After stable saline baselines were achieved, JWS was dissolved in sterile saline and administered IP 15 min before testing in the DSDT method. The doses administered were: 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 mg/kg (corresponding to a range of 0.15 to 29.6 μmoles/kg). Each dose was fully randomized among the rats as to order of administration and repeated 1–2 additional times in all animals. Dose–effect curves were then constructed and later, in a separate set of experiments, the optimal dose (that which produced the greatest improvement over all three delays in each individual animal) was repeated 1–2 additional times for each animal (after a 2-week drug washout).

Statistical Analyses

Passive (inhibitory) avoidance

Testing latencies (latencies to cross into the unsafe side) were analyzed by the nonparametric method of Kruskal-Wallis (analysis of variance on ranks) with post hoc comparisons made according to Dunn's method. Learning frequencies (number of animals not crossing vs. those crossing into the unsafe side) were compared using chi-square analyses (2×4 contingency table for comparisons across all groups and post hoc 2×2 contingency table analysis for individual group comparisons).

Water maze studies

During maze acquisition, the data were collapsed across trials for each day and averaged to obtain a mean performance for each animal. Two-way analysis of variance (ANOVA) with repeated measures was used to compare daily group performance (latencies). One-way ANOVA was used to compare group performance of probe trials and performance of the visual acuity test. All post hoc analyses were performed using the Student-Newman-Keuls method.

DSDT

Dose effects, delay effects, and dose × delay interactions were analyzed using a two-way repeated measures ANOVA. Drug responses were compared to the

closest previous saline administration. Repeated optimal dose administration was analyzed for treatment, delay, and treatment × delay effects using a two-way repeated measures ANOVA and response latencies associated with drug treatments were analyzed by a one-way repeated measures ANOVA. All post hoc comparisons for DSDT studies were made according to the Student-Newman-Keuls method. In all of the above statistical comparisons, a P value < 0.05 was considered significant.

Receptor Binding Experiments

Membrane preparations

Crude membrane preparations were made from rat cerebral cortices as follows: rats were sacrificed by decapitation, the brains rapidly removed, rinsed, and immersed in ice-cold buffer: 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2 mM $CaCl_2$, (pH 7.0). The cortex was subsequently dissected and homogenized for 1 min in 4 ml of buffer using a Bellco glass homogenizer with Teflon pestle. The homogenate was then centrifuged at 49,000*g* for 20 min at 4°C. The supernatant was decanted, the pellet resuspended in 4 ml of incubation buffer, and the process repeated two additional times. The resulting pellets were resuspended in incubation buffer to provide a final protein concentration of 2 mg/ml. Protein concentrations were determined using the Bio-rad protein assay system (Bio-Rad Laboratories, Hercules, CA) and bovine serum albumin was used as the standard.

Binding assay conditions

[^{3}H]-Cytisine binding to nicotinic receptors was estimated in 12 × 75 mm polystyrene tubes in incubation buffer (see above) in a final volume of 250 µl. Saturation studies were performed with [^{3}H]-cytisine concentrations ranging from 0.05–15 nM in order to obtain ligand K_d. Standard displacement assays were subsequently performed with JWS or nicotine dissolved in deionized water and added to the tubes at the indicated concentrations prior to the addition of [^{3}H]-cytisine. The tissue sample (protein) quantity and [^{3}H]-cytisine concentration were 500 µg and 1 nM, respectively. Nonspecific binding was determined using membrane samples incubated in parallel in the presence of 10 µM nicotine (which was added prior to [^{3}H]-cytisine). After all assay components were added, the tubes were incubated at 4°C for 75 min. All assays were performed in duplicate.

Filtration conditions

After incubation, the reaction mixture was filtered through glass fiber filters (Schleicher and Schuell #32) and washed three times with 3 ml of ice-cold buffer using a Brandel Cell Harvester (Gaithersburg, MD). The filters were then dried and placed in scintillation fluid for at least 4 h prior to scintillation counting. Membrane-bound radioactivity was then quantitated after 4 h in a scintillation counter (Beckman model LS 6000TA, Beckman Instrument, Schaumburg, IL) with counting efficiency ~45%.

Binding data analysis

The data points derived from the specific binding (B) of [^{3}H]-cytisine were fit by nonlinear regression analysis (SigmaPlot, Jandel Scientific, Corte Madera, CA) to the mass action expression for ligand binding to a single population of noninteracting sites: $B = B_{max} * [C]/K_d + [C]]$, where B_{max} is the maximum number of binding sites, $[C]$ is the concentration of radioligand and K_d is the equilibrium dissociation constant. Binding of nicotine or JWS to nicotinic receptors was subsequently inferred from their ability to displace the specific binding of [^{3}H]-cytisine. K_I values were calculated from the equation: $K_I = IC_{50}/(1+[L]/K_d)$ where $[L]$ is the concentration of radioligand, as suggested by Cheng and Prusoff [1973].

RESULTS

Passive (Inhibitory) Avoidance

Median step-through latencies to enter the dark chamber and learning frequencies during retention trials after the various drug treatments are illustrated in Figure 2. Highly significant treatment effects were apparent for both the latency data (Kruskal-Wallis test, $H = 29.5$, $P < 0.0001$) and the frequency data (chi-square analysis, $\chi^2 = 31.8, P < 0.001$). Post hoc analyses revealed that scopolamine 0.5 mg/kg, compared to saline, decreased the median latency to cross (and reduced the frequency of animals which learned not to cross) into the unsafe portion of the cage ($P < 0.05$). JWS 0.5 mg/kg when combined with scopolamine 0.5 mg/kg did not significantly reverse these effects of scopolamine ($P > 0.05$). In contrast, JWS 1.0 mg/kg when combined with scopolamine 0.5 mg/kg returned median latency to control levels and significantly reduced the frequency of crossings from scopolamine levels ($P > 0.05$).

Water Maze Experiments

Hidden platform test

The latencies to locate a hidden platform in the water maze for days 1–8 are illustrated in Figures 3 and 4. Statistical comparisons across all eight groups revealed the following results: group effect, [$F(7,48) = 39.8$, $P < 0.0001$]; day effect, [$F(7,49) = 93.6, P < 0.0001$]; group × day interaction, [$F(336,447) = 2.74$, $P < 0.0001$]. Under saline conditions the rats learned to locate the hidden platform with progressively shorter latencies until day 6, when asymptotic levels of performance were reached. Post hoc comparisons indicated that each of the JWS doses tested significantly reduced mean latencies on day 2 (designated by * in Fig. 3, $P < 0.05$) compared to saline

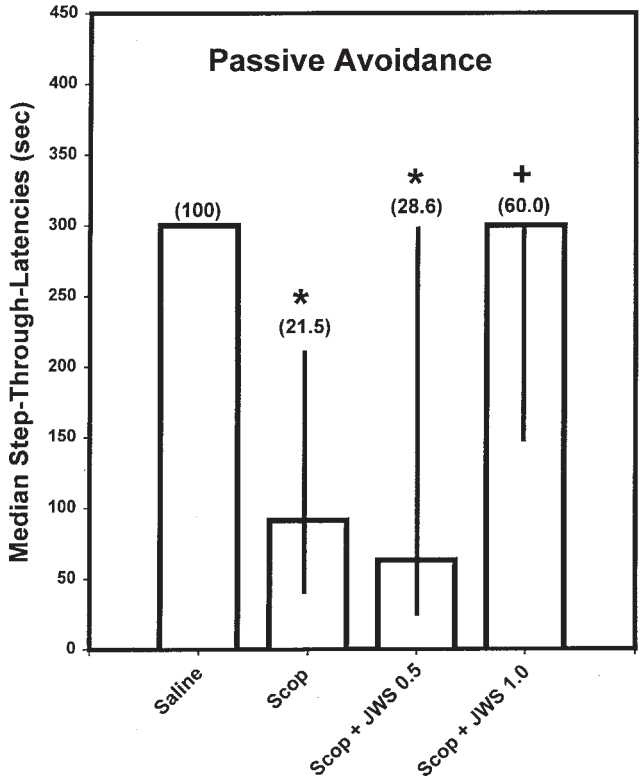


Fig. 2. Effects of saline, scopolamine 0.5 mg/kg, or the combination of scopolamine and either 0.5 or 1.0 mg/kg of JWS on 48-h passive (inhibitory) avoidance training. Saline = saline + saline; scop = scopolamine 0.5 mg/kg + saline; scop + JWS = scopolamine 0.5 mg/kg + JWS at the doses indicated. Bars represent median step-through latencies (in seconds) to enter the dark chamber and the range between the 25th and the 75th percentiles (vertical lines). The numbers in parentheses represent learning frequencies (% of rats having learned not to cross into the unsafe portion of the chamber). * = significantly different ($P < 0.05$) from saline performance, + = significantly different ($P < 0.05$) from scopolamine performance.

controls. No significant differences were observed between the saline group and any of the JWS (alone) groups on the remaining 6 days of testing. Scopolamine 0.5 mg/kg significantly impaired platform location; in fact, asymptotic levels of performance were not reached after 8 days of testing. None of the doses of JWS significantly reversed the scopolamine-induced learning deficits, although a trend toward improved performance was associated with the 0.1 mg/kg dose on days 6–8 ($P = 0.07$).

Probe trial

Drug effects on "Spatial Bias" indicated by the percent of time spent in the quadrant where the platform had been located during the first 8 days of testing are illustrated in Figure 5. Highly significant drug effects were observed, [$F(7,47) = 6.68, P < 0.0001$] and post hoc analyses revealed that the 0.1 and 0.5 mg/kg doses of JWS increased the bias for the correct quadrant significantly over

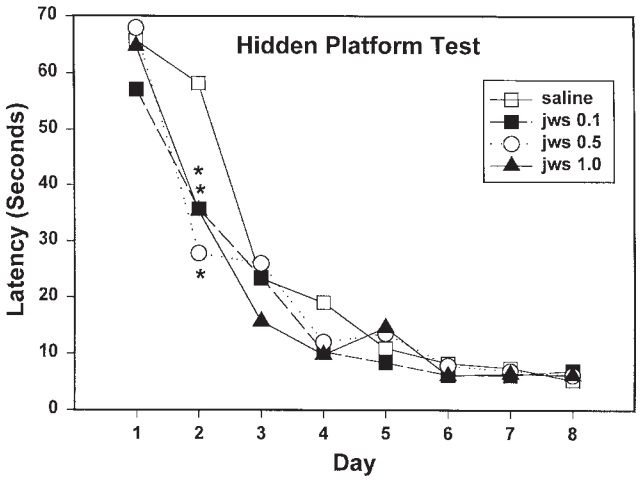


Fig. 3. Mean latencies to locate a hidden platform on each day of water maze training by 1-year-old rats administered either saline or JWS in doses of 0.1, 0.5, or 1.0 mg/kg. * = significantly different ($P < 0.05$) from saline performance.

saline performance levels. The 1.0 mg/kg dose of JWS also increased performance; however, the improvement did not reach the required level of significance ($P > 0.05$). Although scopolamine (0.5 mg/kg) reduced the percent of time in the target quadrant when compared to saline, there was no statistically significant difference ($P > 0.05$) and the combination of JWS (at the three doses tested) combined with scopolamine did not significantly alter this effect.

Visual platform test

Significant drug effects, [$F(7,47) = 2.88, P = 0.014$] were also observed when the ability of rats to locate a highly visible escape platform was assessed. The results are rep-

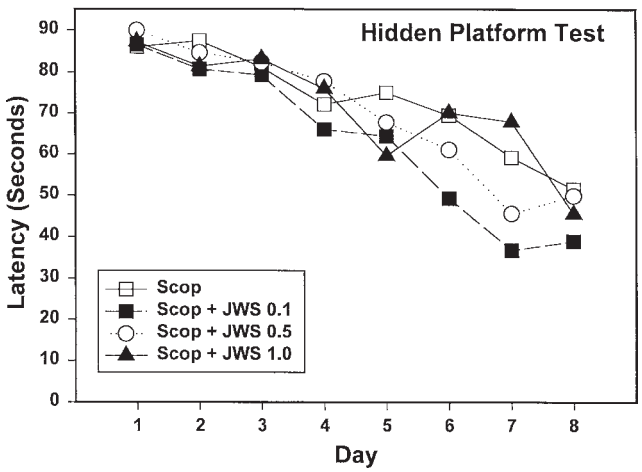


Fig. 4. Mean latencies to locate a hidden platform on each day of water maze training by 1-year-old rats administered either scopolamine 0.5 mg/kg, or the combination of scopolamine 0.5 mg/kg and JWS in doses of 0.1, 0.5, or 1.0 mg/kg.

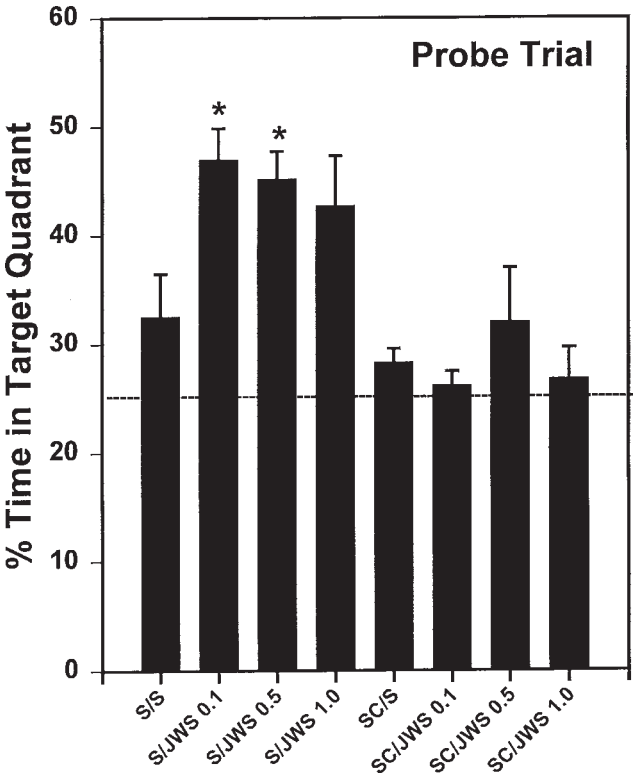


Fig. 5. Performance of the water maze probe trial by 1-year-old rats administered saline + saline (S/S); saline + JWS (S/JWS) in doses of 0.1, 0.5, or 1.0 mg/kg; scopolamine 0.5 mg/kg + saline (SC/S); or the combination of scopolamine 0.5 mg/kg and JWS (SC/JWS) in doses of 0.1, 0.5, or 1.0 mg/kg. Each bar represents the percentage of the total time (±SEM) spent in the quadrant where the platform was located in the previous 8 days of testing. * = significantly different ($P < 0.05$) from saline performance.

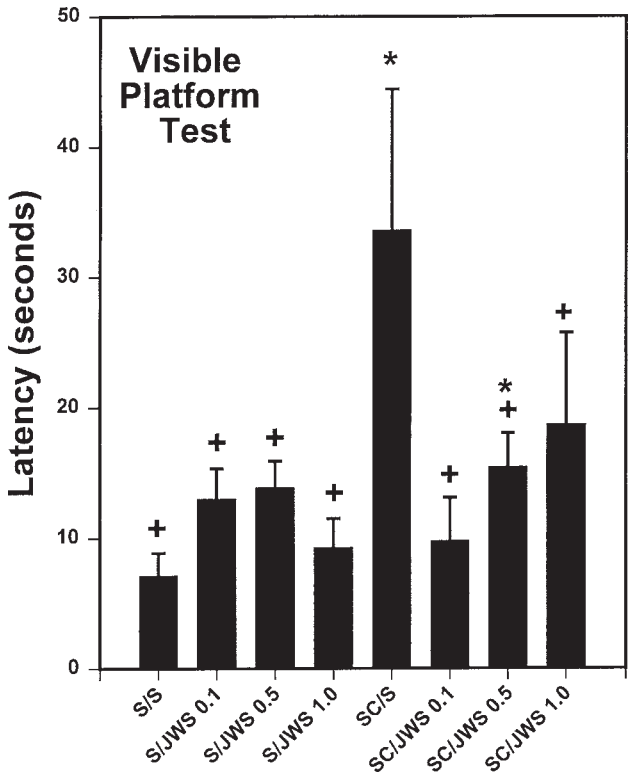


Fig. 6. Latency (sec) to locate a highly visible platform by 1-year-old rats administered saline + saline (S/S); saline + JWS (S/JWS) in doses of 0.1, 0.5, or 1.0 mg/kg; scopolamine 0.5 mg/kg + saline (SC/S); or the combination of scopolamine 0.5 mg/kg and JWS (SC/JWS) in doses of 0.1, 0.5, or 1.0 mg/kg. Each bar represents the latency in seconds (±SEM). * = significantly different ($P < 0.05$) from saline (S/S) performance; + = significantly different ($P < 0.05$) from scopolamine (SC/S) associated performance.

resented in Figure 6. Post hoc analyses revealed that scopolamine (0.05 mg/kg) produced significant impairments (average ~30 sec to locate the platform compared to ~8 sec under baseline conditions) and that each of the doses of JWS significantly reduced this effect of scopolamine. JWS administered alone (at each of the three doses) did not significantly affect visible platform location.

DSDT

JWS dose–effect

The effects of JWS on DSDT accuracy are represented in Figure 7, boxes 1–3, dose effect [$F(5,11) = 4.13$, $P = 0.0002$]; and delay effects [$F(2,10) = 11.47$, $P = 0.003$], dose × delay interaction [$F(22,110) = 1.10$, $P = 0.38$]. Post hoc analysis revealed that no statistically significant improvements were rendered across delays and dose and, in fact, a significant decrement in performance was produced at the 10 mg/kg dose.

JWS optimal dose

Figure 7 (box 4) indicates DSDT accuracy by delay after JWS optimum dose readministration: significant improvements in accuracy were observed, treatment effects [$F(1,5) = 15.44, P = 0.011$]; delay effects [$F(2,10) = 2.19, P = 0.16$]; treatment × delay interaction [$F(2,10)] = 6.33$, $P = 0.017$]. Post hoc comparisons indicated that DSDT improvements in accuracy ($P < 0.05$) were rendered after JWS administration at the medium delays, from a saline value of 70.7 ± 1.5 to 79.4 ± 1.9 percent correct, corresponding to a 13.6% improvement above baseline over the 64 trials/session, and at long delays from 67.8 ± 1.3 to 72.8 ± 1.8 percent correct, corresponding to a 7.8% improvement over the 64 trials/session.

DSDT latencies

The effects of the various doses of JWS on DSDT choice latencies are represented in Table 1. There was a significant treatment effect [$F(5,36) = 3.52$, $P = 0.009$] and post hoc analysis indicated that the 10 mg/kg dose of JWS significantly ($P < 0.05$) increased choice latency.

Receptor Binding Experiments

Nicotine and JWS displaced [^{3}H]-cytisine from rat cortical synaptosomes in a concentration-dependent man-

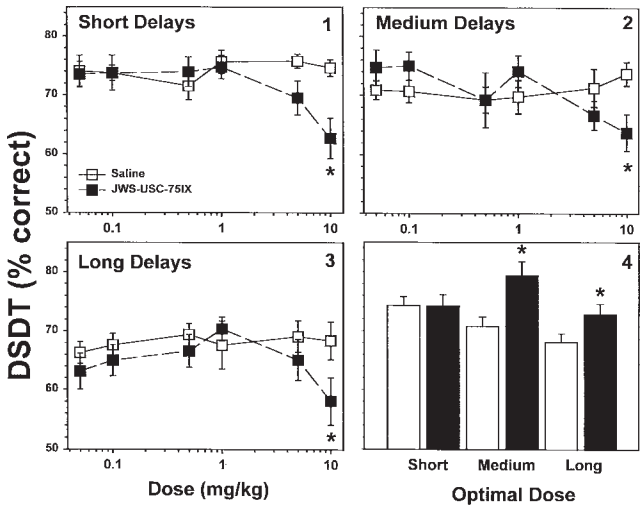


Fig. 7. Boxes 1–3: Dose–effect curves for JWS at each delay in the rat DSDT method. Each point represents the mean (±SEM) of 2–3 replicates per animal (six rats) for each dose. Testing was initiated 15 min following IP injection. Box 4: Performance of the DSDT method at each delay interval (±SEM) by six rats, 15 min following the individualized optimal dose of JWS. * = significantly different from baseline (saline) performance.

ner. Mean K_I values were 8.7×10^{-9}M and 3.7×10^{-4}M for nicotine and JWS, respectively. Thus, nicotine exhibited a relatively high affinity for this binding site, as indicated by the low (nM) K_I value for [^{3}H]-cytisine displacement, whereas JWS had considerably lower affinity.

DISCUSSION

While considerable cholinergic degeneration is known to occur in AD, some function remains even in the very advanced stages of the disease [for review, see Quirion et al., 1995]. At least a proportion of muscarinic receptor populations appear to be functional and some ability to synthesize and release acetylcholine is retained in postmortem cortical and hippocampal tissues from AD patients. [Nilsson et al., 1987; Pearce and Potter, 1991]. It thus appears that the development of novel compounds designed to optimize synaptic acetylcholine levels through autoreceptor activity or other means is important. In the present study, JWS, a compound with combined AChEI and M_2 antagonist activity (in vitro), was thus evaluated in a series of behavioral experiments.

In inhibitory avoidance experiments, JWS (1.0 mg/kg) clearly improved scopolamine-induced deficits in learning to avoid an unsafe region of the shuttle box, as evidenced by both latency and learning frequency analysis. In water maze experiments, JWS (at the three doses tested) significantly improved "spatial navigation learning" as evidenced by the ability of 1-year-old rats to locate a hidden platform on day 2 of training. As observed in numerous previous experiments in younger rats under saline conditions, day 2 is usually the day in which the largest decrease in latencies occur. JWS (at two of the doses tested) also significantly improved "spatial bias" for the previous platform location when tested on day 9. The compound was not particularly effective at reducing scopolamine-induced deficits in this task. As indicated in Figure 6, scopolamine did appear to affect vision in these rats, as indicated by the prolonged time to find a clearly visible platform. JWS did significantly reduce this effect of scopolamine, however. The dose of scopolamine used (0.5 mg/kg) is commonly used in water maze experiments to produce amnestic effects. It is possible that in our study the age of the rats may have rendered them more susceptible to the visual effects of scopolamine.

In the more complex DSDT method, JWS failed to show statistically significant improvements across doses and delays after all the doses were administered and repeated several times. In fact, the 10 mg/kg dose produced significant decrements in DSDT accuracy. We observed, however, that individual rats appeared to respond (with improved accuracy) on an individual basis to certain doses (in some cases with quite robust improvements). We subsequently selected the optimal dose for each rat and repeated this dose on several additional occasions in separate experiments after a minimum 2-week washout from dose–effect studies. Repeated optimal dose administration significantly improved performance (accuracy) of the DSDT method at the medium and long, presumably more difficult delay, intervals. The fact that statistically significant improvements in performance remained after several repeats of the optimal dose indicates a lack of rapidly developing tolerance to the effects. With the exception of the highest dose of JWS tested (10.0 mg/kg) in the DSDT method, no significant effects on lever choice latencies were observed. This indicates that sedative or stimulant properties were unlikely to have contributed to the enhanced performance of the task observed after optimal dose readministration. At the 10.0 mg/kg dose, increased choice latencies were observed, although no visible signs

TABLE 1. DSDT Lever Choice Latencies

Treatment	Dose (MG/KG)	Latency (SEC)
Saline	—	0.95 ± 0.15
JWS-USC-75IX	0.05	0.80 ± 0.11
	0.1	0.87 ± 0.18
	0.5	1.08 ± 0.26
	1.0	0.88 ± 0.11
	5.0	1.33 ± 0.28
	10.0	6.45 ± 2.90*

Data represent mean choice latencies ± SEM for lever selection in the DSDT task 15 min after the administration of saline or various doses of JWS-USC-75IX.

*Significant difference ($P < 0.05$) from the saline (baseline) value.

of toxicity (i.e., cholinergic effects—salivation, excessive urination, defecation, muscle fasiculations, etc.) were observed in any of the animals at any of the doses. Thus, the underlying basis for the detrimental effects at the 10 mg/kg dose are unclear at this time. It is interesting to note that the compound has weak antagonistic activity at the M_1 cholinergic receptor, IC_{50} ~1.0 μM [Valli et al., 1992] which could contribute to the performance decrements observed at this dose.

Finally, JWS did not appear to have significant nicotinic receptor binding activity, as indicated by the relatively high (μM) K_I obtained in [^{3}H]-cytisine displacement assays as compared to nicotine (a standard of comparison), which demonstrated nM affinity. Therefore, the compound does not appear to express its behavioral effects through nAChr mechanisms (at least via the $\alpha_4\beta_2$ subtype). The binding results with nicotine in rodent brain are in general agreement with the data presented by our group previously [Terry et al., 1998] and by other investigators [Reavill et al., 1988; Grady et al., 1992].

Since JWS was derived from the ranitidine molecule (a histaminic H_2 antagonist), it is certainly conceivable that the compound may have activity at histaminic receptors. Several studies in the past several years (with histamine receptor ligands) have indicated that the endogenous autacoid, histamine, may also play an important role in learning and memory, as well as a number of other functions in the CNS [Smith et al., 1994; Miyazaki et al., 1995]. Therefore, studies to further elucidate the biochemical effects as well as behavioral effects of JWS (and related compounds) are warranted.

In summary, the data obtained in this study indicate moderate improvements in learning and memory in young and middle-aged rodents of two strains across three separate behavioral models after administration of JWS. These results appear to substantiate the hypothesis that combining AChEI activity with M_2 cholinergic receptor antagonism offers one potential means of improving cognition. The fact that JWS possesses both of these pharmacological actions in one molecule and exhibits a low toxicity profile [Valli et al., 1992] provides additional support for the potential use of this or similar agents in disorders of memory which involve cholinergic dysfunction.

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