The selective $M_1$ muscarinic cholinergic agonist CDD-0102A enhances working memory and cognitive flexibility


Department of Psychology, Laboratory of Integrative Neuroscience, University of Illinois at Chicago, Chicago, IL, USA (MER, SA, AS); Mithridion, Inc., 505 Science Drive, Suite C, Madison, WI 53711 (TMT, JEB; Departments of Pharmacology and Medicinal & Biological Chemistry, The University of Toledo, Toledo, OH, USA (WSM)
Running Title Page

Running Title: CDD-0102A, M₁ muscarinic agonist, enhances cognition

Corresponding Author: William S. Messer, Jr., Ph.D., Department of Pharmacology, College of Pharmacy and Pharmaceutical Sciences, The University of Toledo, 3000 Arlington Avenue, MS #1015, Toledo, OH 43614; Phone: (419) 383-1958; Fax: (419) 383-1909; Email: william.messer@utoledo.edu

Number of Text Pages: 19
Number of Tables: 0
Number of Figures: 5
Number of Words in Abstract: 249
Number of Words in Introduction: 453
Number of Words in Discussion: 1059
Number of References: 29
Section Assignment: Neuropharmacology

Abbreviations:

5-(3-ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine hydrochloride (CDD-0102A)
ABSTRACT

Various neurodegenerative diseases and psychiatric disorders are marked by alterations in brain cholinergic function and cognitive deficits. Efforts to alleviate such deficits have been limited by a lack of selective M₁ muscarinic agonists. CDD-0102A is a partial agonist at M₁ muscarinic receptors with limited activity at other muscarinic receptor subtypes. The present studies investigated the effects of CDD-0102A on working memory and strategy shifting in rats. CDD-0102A administered i.p. 30 minutes before testing at 0.1, 0.3 and 1 mg/kg significantly enhanced delayed spontaneous alternation performance in a four-arm cross maze, suggesting improvement in working memory. In separate experiments CDD-0102A had potent enhancing effects on learning and switching between a place and visual cue discrimination. Treatment with CDD-0102A did not affect acquisition of either a place or visual cue discrimination. In contrast, CDD-0102A at 0.03 and 0.1 mg/kg significantly enhanced a shift between a place and visual cue discrimination. Analysis of the errors in the shift to the place or shift to the visual cue strategy revealed that in both cases, CDD-0102A significantly increased the ability to initially inhibit a previously relevant strategy and maintain a new, relevant strategy once selected. In anesthetized rats, the minimum dose required to induce salivation was approximately 0.3 mg/kg i.p. Salivation increased with dose, and the estimated ED₅₀ was 2.0 mg/kg. The data suggest that CDD-0102A has unique memory and cognitive enhancing properties that might be useful in the treatment of neurological disorders at doses that do not produce adverse effects such as salivation.
INTRODUCTION

Five subtypes of muscarinic receptor have been identified in mammals. The odd numbered receptor subtypes (M₁, M₃ and M₅ receptors) stimulate phosphoinositide metabolism through the G₁₁ family of G proteins, while the even numbered receptors (M₂ and M₄ receptors) inhibit adenylyl cyclase activity through the Gᵢₒ family of G proteins. Muscarinic receptors play important roles in regulating peripheral responses to acetylcholine in the parasympathetic nervous system. For example, M₂ receptors slow the heart rate and decrease the force of cardiac contractions, while M₃ receptors stimulate gastrointestinal activity and promote secretion from salivary and lacrimal glands. Although salivation has been attributed to activation of M₃ receptors, it should be noted that M₁ receptors also may contribute to the effects of muscarinic agonists on the salivary glands (Gautam et al., 2004).

Studies with knockout mice have provided some insight into the potential roles of muscarinic receptor subtypes, as reviewed previously (Wess, 2004; Wess et al., 2007). Studies with knockout mice suggest that both M₁ and M₂ receptors are involved in memory function. M₁ receptors are critical for performance of non-matching-to-sample working memory tasks, (Anagnostaras et al., 2003) while M₂ receptors play important roles in both working memory and behavioral flexibility (Seeger et al., 2004). Muscarinic receptors also modulate biochemical signaling pathways including extracellular signal-regulated kinase (ERK) (Berkeley et al., 2001) and neurophysiological responses associated with memory function such as long term potentiation (LTP) and long-term depression (LTD) (Berkeley et al., 2001; Shinoe et al., 2005; Origlia et al., 2006). The role of muscarinic receptor subtypes in memory and cognitive function likely depends on
a number of factors, including localization of receptors in particular brain regions, the
signaling pathways activated (or inhibited) by each receptor subtype and the type of
memory being assessed. Overall however, there is strong evidence that activation of
M₁ receptors results in enhanced memory function, leading to drug development efforts
focused on M₁ agonists for the treatment of memory and cognitive deficits associated
with neurological disorders including Alzheimer’s disease and schizophrenia.

5-(3-Ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine hydrochloride (CDD-
0102A) was synthesized and characterized as a selective muscarinic agonist in a
variety of binding and functional assays (Dunbar et al., 1993; Messer et al., 1997a;
Messer et al., 1997b). CDD-0102A is a functionally-selective agonist with partial
agonist activity at M₁ muscarinic receptors, weak activity at M₃ receptors and no activity
at other muscarinic receptor subtypes (see Figure 1). In the studies described here, the
compound was evaluated further for its ability to enhance memory function and
behavioral flexibility in rats. Adverse effects were assayed by measuring the effects of
CDD-0102A on saliva production in anesthetized rats at comparable doses. The
findings suggest that CDD-0102A may be useful in alleviating memory and cognitive
flexibility deficits in various neurological disorders.

METHODS

Animals

For behavioral studies, male Long-Evans rats were purchased from Harlan
(Indianapolis, IN) weighing between 325-375 g. Rats were individually housed in plastic
cages (20.3 cm x 20.3 cm x 41.9 cm) in an environmentally controlled room (23°C, 30% humidity) on a 12h:12h light:dark cycle (lights on at 7:00am). After at least four days to acclimate to the colony room, rats were handled for 10 minutes per day for four days to adjust to being handled during training and testing. At the same time rats began to be handled, rats were also food restricted to reduce their weight to 85-90% of their ad libitum weight. Rats had free access to water throughout the experiment. Animal care and use conformed to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Institutional Laboratory Animal Care and Use Committee at the University of Illinois at Chicago.

For studies of salivation, male Long-Evans rats were purchased from Charles River Laboratories (Wilmington, MA) weighing between 250-425 g. Animals were kept in a vivarium on a 12-h light/dark cycle. Humidity was maintained at 30-70%, and temperature was maintained at 20-24°C. All procedures were approved by the Institutional Animal Care and Use Committee at Mithridion, Inc.

**Behavioral Testing**

All behavioral testing occurred in a cross maze made of translucent black acrylic. Each arm measured 55 cm long, 10 cm wide, and 15 cm in height. A circular food well, with a diameter of 3.2 cm and a depth of 1.6 cm was placed 3 cm from the end of each arm. The maze was placed on a table with a height of 72 cm.

*Delayed Spontaneous Alternation.* In this test a four-arm cross maze was used as described above. This test did not contain any food rewards, but took advantage of a rat’s natural tendency to spontaneously alternate or choose the least recently visited
location (Dember and Fowler, 1958). Consistent with the idea that this test has a mnemonic component, increasing the delay between arm choices (Ragozzino et al., 1996; Mohler et al., 2007) or increasing the number of locations to choose (Ragozzino et al., 1995; Ragozzino et al., 1996) reduces alternation scores. For the test, each rat was placed in the testing room 5 minutes prior to behavioral testing. At the beginning of a session, a rat was allowed to freely choose an arm, but after making a choice, a block was placed into that arm for 30 seconds preventing a rat from entering another arm. The block was a 21.5 cm x 12 cm piece of plastic. After the 30 second delay, the block was removed and a rat was allowed to enter another arm. The number and sequence of arm entries was recorded. An arm entry was recorded when all four paws entered an arm. An alternation was defined as entry into four different arms on overlapping quadruple sets of arm entries; e.g., a quadruple set consisting of arm choices A, D, C, B was an alternation but a quadruple set consisting of arm choices A, D, A, C was not. The percent alternation score is equal to the ratio of (actual alternations/possible alternations) multiplied by 100; chance performance on this task is 22.2%. The total number of possible alternations was equal to the total number of arm choices minus three. For example, a rat that made the following sequence of arm choices in a session: A,C,D,A,B,C,B,D,A,B,C,D would have nine possible alternations. In this example, a rat made five alternations (represented in bold) in which there were four different arm entries in consecutive sets of four arm choices. Thus, a rat would have an alternation score of 55.6%. The test session lasted 15 minutes. Rats that made less than 11 arm entries were excluded from the analysis. This criterion is similar to that used in previous experiments (Ragozzino et al., 1996; Mohler et al., 2007). Thirty minutes before testing,
a rat received an i.p. injection of one of the following treatments: 1) saline (n = 8); 2) CDD-0102A 0.03 mg/kg (n = 8); 3) CDD-102A 0.1 mg/kg (n = 8); 4) CDD-0102A 0.3 mg/kg (n = 7) or 5) CDD-0102A 1 mg/kg (n = 8). CDD-0102A was mixed in sterile saline.

**Place - Visual Cue Discrimination Learning and Strategy Switching.** A different group of rats was used for the discrimination learning and strategy switching tests from that used in the delayed spontaneous alternation test. Prior to testing, rats were food-restricted to maintain their weight at 85% of their free-feed weight, handled and trained in the maze to obtain half piece of Froot Loops™ cereal in the cross-maze. A similar training procedure was used as described previously (Ragozzino et al., 2002a; Ragozzino et al., 2009).

Following training, each rat was tested across two consecutive days. In the first experiment each rat had to learn a place discrimination. Rats were started in a pseudorandom manner from one of two different arms such that any start arm was not used more than 3 consecutive trials. The two start arms (“east” and “west”) were always opposite each other. A black plastic block was placed in the entrance of the maze arm opposite to that of the start arm, giving the maze a T-shape. Thus, the same two choice arms were used no matter what start arm was used. A rat was started in the stem arm with only one of the two choice arms baited. In the acquisition phase, one choice arm or place was designated the reinforced arm which contained a ½ piece of cereal reinforcement on each trial. In this phase, a rat was required to enter the reinforced arm containing a ½ piece of cereal. If a rat chose the correct arm, the trial was terminated after a rat consumed the cereal piece. If a rat chose the incorrect arm, the trial was
terminated after a rat reached the unbaled food well. Black and white visual cues that lined the base and side walls of the choice arms were assigned on a pseudorandom basis to be on the left or right of the start arm so that they occurred in each start arm an equal number of times in blocks of 12 trials. Between trials, the maze, visual cues, and block were wiped down with 2% Quatricide® solution to minimize the animals' ability to use odor cues for discriminations. Between trials, a rat was placed in a holding chamber which was placed on a table next to the maze. The maze was then wiped down and re-baited if necessary. The inter-trial interval was approximately 15 seconds. To minimize the use of intramaze cues the maze was rotated 90° every fourth trial. The criterion for acquisition of the place discrimination was 10 consecutive correct trials.

The day following the acquisition phase, each rat was tested on the switch to the visual cue strategy. In the switch phase, a rat was required to switch strategies from always entering a choice arm based on spatial location to entering a choice arm based on visual cue (black or white). Again, the visual cues were switched in a pseudorandom manner between choice arms so that a particular cue was associated with the same choice arm for a maximum of three consecutive trials and was equally associated with each turn direction across consecutive blocks of 12 trials. The learning criterion in the switch phase was also 10 consecutive correct arm choices. Thirty minutes prior to each test phase, rats received an i.p. injection of the designated treatment for that phase. The rats were divided into the following groups: 1) saline - saline (n = 9); 2) saline - CDD-0102A 0.003 mg/kg (n = 7); 3) saline - CDD-0102A 0.03 mg/kg (n = 9); 4) vehicle – CDD-0102A 0.1 mg/kg (n = 8); 5) CDD-0102A 0.03 mg/kg-saline (n = 8); or 6) CDD-0102A 0.01 mg/kg-saline (n = 7).
In a separate experiment, the effect of CDD-0102A on initial learning of a visual cue strategy and switch to a place strategy was determined. All other aspects of the testing procedure were as described above. The treatment groups in this experiment were as follows: 1) saline - saline (n = 8); 2) saline - CDD-0102A 0.003 mg/kg (n = 7); 3) saline - CDD-0102A 0.03 mg/kg (n = 7); 4) vehicle – CDD-0102A 0.1 mg/kg (n = 8); 5) CDD-0102A 0.03 mg/kg-saline (n = 7); or 6) CDD-0102A 0.01 mg/kg-saline (n = 8).

In the switch phase of each experiment, the errors committed were separated into different categories and analyzed. The errors were separated into perseverative, regressive and never-reinforced errors as described previously (Ragozzino et al., 2002b; McCool et al., 2008). In the place acquisition and shift to visual cue experiment, for half the trials a rat had to enter the arm that was in the opposite place as the arm that was reinforced in the place acquisition phase. For example, a rat might have to learn to always enter the “north” arm in the place acquisition phase. In the shift to visual cue phase, a rat now had to always enter the arm with the black visual cue. Thus, when the black visual cue was in the “south” arm, a rat had to switch from choosing the “north” arm and enter the “south” arm. These trials were used to analyze the perseverative and regressive errors. These trials were initially separated into consecutive blocks of 4 trials each. Perseveration was defined as initially entering the incorrect arm in three or more trials per block. Thus, if a rat was initially choosing the previously correct choice on the majority of trials it was considered perseveration. Once a rat made less than three errors in a block the first time, all subsequent errors were no longer counted as perseverative errors. When perseveration ended, as defined above, the number of errors was counted when a rat reverted back to the previously correct
choice on those trials that required the opposite turn as on the place version. These errors are referred to as regressive errors. During the shift, a third type of error could be made if a rat entered the arm that contained neither the presently correct visual cue nor the previously correct location. For example, in half the trials the presently correct visual cue (e.g. black) was in the previously correct spatial location (e.g. “north” arm) and if a rat chose the “south” arm this would count as an error. These errors are referred to as never-reinforced errors because a rat was never reinforced for this choice on either acquisition or switch phases.

These same types of errors were calculated in a similar manner for the experiment in which rats first learned a visual cue strategy and then switched to a place strategy.

Statistical Analysis

In the spontaneous alternation task, a one-way analysis of variance was used to identify differences in percent alternation scores and number of arm entries across groups. Newman-Keuls post-hoc tests were used to compare treatment and control group measures. In the discrimination learning tests, one-way analyses of variance were used to identify differences in trials to criterion for acquisition and strategy switching. Separate analyses of variance were carried out to determine group differences in perseverative, regressive, and never-reinforced errors. Newman-Keuls post-hoc tests were used to compare differences between treatment groups.
Salivation studies

Salivation was quantified in rats following i.p. administration of CDD-0102A. Rats were first anesthetized with isoflurane, and then dosed with CDD-0102 by i.p. injection. Anesthesia was maintained by a nose cone that delivered 1.5-2.5% isoflurane in 500 ml/minute of oxygen. CDD-0102A was dissolved in phosphate-buffered saline and the injection volume was 2 ml/kg. The hind limbs of anesthetized animals were taped to the top of an incline (25° grade) so that the animal was oriented with its head near the bottom of the slope and its dorsal-surface oriented upward. Body temperature was monitored by a rectal thermometer, and maintained at 36-38°C by a heating pad. The animal’s nose was inserted into a nose cone for maintaining anesthesia while the entire mouth remained outside this nose cone. Pre-weighed filter paper (approximately 5x5 cm) was placed underneath the mouth to absorb the draining saliva. After 5 minutes, the saliva-wetted filter paper was weighed and replaced with fresh, pre-weighed filter paper. Total salivary output was calculated by subtracting the mass of the filter paper from the combined mass of paper plus saliva as collected over 60 minutes.

RESULTS

Delayed Spontaneous Alternation

Treatments with CDD-0102A significantly enhanced delayed spontaneous alternation in a dose-dependent manner (see Figure 2A). An ANOVA revealed there was an overall group effect, $F(4, 34) = 8.59$, $P < 0.01$. Post-hoc tests indicated that CDD-0102A at 0.3 and 1.0 mg/kg significantly enhanced alternation performance
compared to that of saline controls and CDD-0102A at 0.03 mg/kg (P values < 0.01). CDD-0102A at 0.1 mg/kg also significantly enhanced alternation performance compared to that of saline controls and CDD-0102A at 0.03 mg/kg (P values < 0.05).

In contrast to alternation scores, CDD-0102A treatment across all doses led to a similar number of arm entries as that of the control group (see Figure 2B). An ANOVA on the number of arm entries among the groups revealed no significant difference, F(4, 34) = 0.28, P > 0.05.

Place Discrimination Acquisition and Switch to Visual Cue Discrimination

Treatment with CDD-0102A at 0.03 or 0.1 mg/kg 30 minutes prior to acquisition of a place discrimination did not affect learning compared to saline-treated rats (see Figure 3A). An ANOVA indicated that there was no significant treatment effect on place acquisition, F(5,42) = 0.51, P > 0.05. However, there was a significant treatment effect on the switch to the visual cue discrimination, F(5,42) = 37.52, P < 0.01. CDD-0102A at doses of 0.03 and 0.1 mg/kg significantly decreased the number of trials to reach criterion as compared to that of all other treatment groups (P values < 0.01). In contrast, performances of rats treated with CDD-0102A at 0.003 mg/kg were similar to those of saline-treated rats (P > 0.05). Thus, CDD-0102A improved a shift to a visual cue discrimination in a dose-dependent manner (see Figure 3B).

The facilitating effects of CDD-0102A on the switch to a visual cue discrimination resulted from a significant decrease in all three of the error measures (see Figures 3C-3E). In particular, CDD-0102A at 0.03 mg/kg significantly reduced perseverative errors compared to that of the lowest dose of CDD-0102A and saline-treated controls (P
values < 0.05). CDD-0102A at 0.1 mg/kg also significantly reduced perseverative errors compared to saline controls and the lowest dose of CDD-0102A (P values < 0.01). CDD-0102A at 0.03 or 0.1 mg/kg also significantly lessened the number of regressive errors as compared to that of the other treatment groups (P values < 0.01). In addition, the number of never-reinforced errors committed were significantly greater in saline-treated rats and CDD-0102A 0.003 treatment group compared to that of CDD-0102A at 0.03 mg/kg (P values < 0.05) and CDD-0102A at 0.1 mg/kg (P values < 0.01).

**Visual Cue Discrimination Acquisition and Switch to Place Discrimination**

The finding that CDD-0102A treatment enhanced performance on a shift to a visual cue discrimination could reflect a facilitation of strategy switching or a more general enhancement of visual cue learning. To determine between these possibilities, an experiment was carried out that examined the effects of CDD-0102A treatment on acquisition of a visual cue discrimination and shift to a place discrimination. All groups, including those receiving CDD-0102A prior to acquisition, performed in a similar manner in learning a visual cue discrimination (see Figure 4A). An ANOVA indicated that there was no significant group effect on visual cue acquisition, F(5,39) = 2.10, P > 0.05. CDD-0102A treatment did lead to an overall group effect in the switch to the place discrimination, F(5,39) = 24.72, P < 0.01. Specifically, CDD-0102A at doses of 0.03 and 0.1 mg/kg significantly enhanced switching to a place strategy as compared to all other treatment groups (P values < 0.01, see Figure 4B). CDD-0102A at 0.003 mg/kg had no significant effect on a switch to a place strategy as compared to controls (P values > 0.05).
CDD-0102A treatment also affected the errors committed in the switch to the place discrimination (see Figures 4C-4E). Compared to all other treatment groups, CDD-0102A at 0.03 and 0.1 mg/kg significantly decreased the number of perseverative errors \((P \text{ values} < 0.01)\) and regressive errors \((P \text{ values} < 0.01)\). In the switch to the place discrimination, each group made very few never-reinforced errors; thus there was no overall group effect on the number of never-reinforced errors committed, \(F(5,39) = 0.83, P > 0.05\).

**Salivation**

CDD-0102A was administered to adult, male, Long-Evans rats at doses of 0.1, 0.3, 1, 2.5, 5 and 10 mg/kg by i.p. injection. Salivary output increased as a function of dose over a 60 minute period as shown in Figure 5. The 0.1 mg/kg dose caused no salivation in rats, while the 5 mg/kg dose produced the most salivation \((4,685 \pm 449 \mu l)\). Salivation from rats dosed with 10 mg/kg was not significantly different from rats given the 5 mg/kg dose \((P > 0.5)\).

**DISCUSSION**

CDD-0102A is a functionally selective M\(_1\) muscarinic agonist with potential utility in treating memory deficits associated with Alzheimer’s disease. In the present studies, acute treatment with CDD-0102A enhanced both delayed spontaneous alternation and strategy switching in a dose-dependent fashion. CDD-0102A facilitated delayed spontaneous alternation without affecting the number of arms choices suggesting that the drug enhanced mnemonic processes without any effect on locomotor activity. This
task requires a short-term or working memory for recent arm choices. Consistent with this idea, inserting a delay between arm choices decreases alternation scores (Ragozzino et al., 1996; Mohler et al., 2007). Previous studies have shown that hippocampal acetylcholine output increases during spontaneous alternation performance and facilitating hippocampal acetylcholine output concomitantly enhances spontaneous alternation performance (Ragozzino et al., 1996; Ragozzino et al., 1998; Mohler et al., 2007). Taken together with the in vivo microdialysis experiments, the present findings suggest that increases in brain acetylcholine release may enhance working memory, in part by activating hippocampal M₁ muscarinic cholinergic receptors.

The finding that CDD-0102A enhanced delayed spontaneous alternation is consistent with other studies demonstrating that M₁-preferring agonists enhance working memory in rodents and non-human primates (Wall et al., 2001; Terry et al., 2002). Moreover, CDD-0102A-enhanced delayed alternation is consistent with previous results indicating that CDD-0102A reverses a working memory deficit induced by an 192-IgG saporin lesion of basal forebrain cholinergic neurons (Messer et al., 2002). However, this is the first demonstration that CDD-0102A alone enhances working memory.

CDD-0102A facilitated strategy switching, regardless of whether a rat switched from a place to visual cue strategy or from a visual cue to a place strategy. In contrast, CDD-0102A treatment did not affect the initial learning of a place or visual cue discrimination. This is the first report of an effect of an M₁-selective muscarinic agonist on strategy switching. Strategy switching was found to be surprisingly sensitive to CDD-0102A, being maximal at a dose of 0.03 mg/kg i.p., a dose lower than that
required to enhance delayed spontaneous alternation, and much lower than that required to cause salivation. The data suggest that a low level of muscarinic receptor occupancy is required for this action. Furthermore, the effects of CDD-0102A are consistent with accumulating evidence that increases in striatal acetylcholine output facilitate cognitive flexibility (Ragozzino and Choi, 2004; Ragozzino et al., 2009), and that this might be mediated by M₁-like muscarinic receptors, because infusions of M₁ muscarinic antagonists into the dorsomedial striatum impair cognitive flexibility (Tzavos et al., 2004; McCool et al., 2008). Because CDD-0102A was administered systemically the drug effect on strategy switching may be due to activation of multiple brain areas and not limited to the striatum.

CDD-0102A enhanced strategy switching by reducing multiple types of errors. In both experiments, CDD-0102A significantly reduced perseverative errors and regressive errors. Thus, CDD-0102A improved the ability to initially suppress a previously learned strategy and/or generate a new strategy as exhibited by reduced perseveration. CDD-0102A also enhanced the ability to maintain a new strategy once selected as observed by a reduction in the number of regressive errors. Several experiments indicate that different prefrontal cortex subregions are critical for the initial inhibition of a previously learned strategy as measured by perseverative errors while manipulation of the dorsomedial striatum selectively affects the ability to reliably execute a new strategy once selected (Ragozzino et al., 2003; Palencia and Ragozzino, 2004; McCool et al., 2008). The prefrontal cortex and striatum are two brain areas that have moderate to high densities of M₁ muscarinic receptors (Levey et al., 1991). Because CDD-0102A treatment reduced both perseverative and regressive errors, the pattern of results
suggests that CDD-0102A may act at both prefrontal cortex and striatal regions to facilitate initial inhibition of a previously relevant strategy while reliably executing a new strategy once selected.

CDD-0102A significantly reduced never-reinforced errors in the shift from the place to visual cue strategy, but did not affect never-reinforced errors in the shift from the visual cue to place strategy. The differential effect is likely due to rats making few never-reinforced errors in the shift to the place. A rat may make a never-reinforced error because it is trying an alternative, incorrect strategy such as reversing the place choice or shifting to a response strategy (e.g. always turn right). The finding that CDD-0102A reduced never-reinforced errors in the shift to the visual cue strategy may indicate that it inhibited the selection of alternative, incorrect strategies and facilitated the selection of the new, correct strategy.

Several muscarinic agonists have been developed, though none approved, for the treatment of Alzheimer’s disease. Liabilities in clinical studies include dose-limiting adverse effects such as nausea, vomiting and diarrhea. In order to further assess the adverse effect profile of CDD-0102A, salivation was measured in rats over a range of doses comparable to those utilized in the behavioral studies. The lowest dose that produced salivation was 0.3 mg/kg i.p. Significant improvement in memory function was observed at doses of 0.1 mg/kg and higher, while doses of 0.03 and 0.1 mg/kg were effective in enhancing behavioral flexibility. The data indicate that CDD-0102A has beneficial effects on memory and cognitive function at doses that do not produce salivation. While salivation has been linked to activation of M₃ muscarinic receptors, it may be that the salivation produced by CDD-0102A in the present studies is due to
activation of M₁ receptors (Gautam et al., 2004). Further studies using, for example, receptor knockout mice are necessary to determine which muscarinic receptor subtypes contribute to the stimulation of salivation by CDD-0102A.

The present findings suggest that CDD-0102A may have a broad range of effects on cognition, facilitating both working memory and cognitive flexibility. Because the present experiment only investigated the effect of acute treatment with CDD-0102A, future experiments examining the effects of chronic treatment on the drug are crucial in determining whether CDD-0102A can possibly serve as an effective treatment for alleviating cognitive deficits. This is particularly important because of the considerable effort in the development of pharmacological agents that selectively act at the M₁ muscarinic acetylcholine receptor, in particular M₁ muscarinic agonists. This is, in large part, because of studies indicating that M₁ muscarinic acetylcholine receptors are altered in Alzheimer’s disease and schizophrenia (Langmead et al., 2008). Both of these conditions are marked by cognitive deficits and thus there has been an interest in developing selective M₁ muscarinic agonists to alleviate the cognitive deficits in these conditions.
Acknowledgements

The authors wish to thank Michael Hendrickson for his help in preparing the manuscript for publication.
Authorship Contributions

Participated in research design: Michael E. Ragozzino, Trevor M. Twose, Joseph E. Beck and William S. Messer, Jr.

Conducted experiments: Michael E. Ragozzino, Sonja Artis, Amritha Singh and Joseph E. Beck

Contributed new reagents: William S. Messer, Jr.


Wrote or contributed to the writing of the manuscript: Michael E. Ragozzino, Trevor M. Twose, Joseph E. Beck and William S. Messer, Jr.
References


Ragozzino ME, Ragozzino KE, Mizumori SJ and Kesner RP (2002b) Role of the dorsomedial striatum in behavioral flexibility for response and visual cue


Footnotes

Dr. William S. Messer, Jr. serves as Chief Scientific Officer and as a consultant to Mithridion, Inc., is an inventor on several patents covering muscarinic agonists and holds a financial interest in the company.

Dr. Trevor Twose is the Chief Executive Officer for Mithridion, Inc. and holds a financial interest in the company.

Dr. Joseph Beck holds a financial interest in Mithridion, Inc.

This research was supported by research grants from the National Institutes of Health [AG02454, AG027951 and P50 HD055751].
LEGENDS FOR FIGURES

Figure 1. Maximal responses of CDD-0102A at muscarinic receptor subtypes. The data are expressed as a percentage of the maximal response produced by the full agonist carbachol for each receptor subtype. Stimulation of phosphoinositide metabolism provided a measure of agonist activity at M₁, M₃ and M₅ receptors expressed in A9 L cells, while inhibition of forskolin-stimulated cAMP formation assessed agonist activity at M₂ and M₄ receptors expressed in A9 L and CHO cells respectively. Data represent the mean (± SEM) from at least three experiments each performed in triplicate. Data for M₁ and M₃ receptors are summarized from previous publications (Messer et al., 1997b).

Figure 2: The effect of CDD-0102A on delayed spontaneous alternation. Each rat received an i.p injection of saline (SAL) or one of four doses of CDD-0102A (CD) 30 minutes prior to a delayed spontaneous alternation test in a 4-arm maze. There was a 30 second delay between each arm choice. A) Mean (± SEM) percent alternation scores. CDD-0102A significantly improved alternation scores at 0.1, 0.3 and 1.0 mg/kg. * = p < 0.05 vs. saline and ** = p < 0.01 vs. saline. B) Mean (± SEM) number of arm choices in delayed alternation test. CDD-0102A did not affect the number arm entries.

Figure 3: The effect of CDD-0102A on place acquisition and switch to a visual cue discrimination. Each rat received an i.p injection of saline (SAL) or one of three doses of CDD-0102A (CD) 30 minutes prior to acquisition and switch phases. The treatments in the legends represent the treatment received prior to acquisition (top row) followed by the treatment received prior to the switch phase (bottom row). A) Mean (± SEM) trials
to criterion on acquisition of a place discrimination. Injection of CDD-0102A had no effect on acquisition. B) Mean (± SEM) trials to criterion on switch to a visual cue strategy. CDD-0102A at 0.03 and 0.1 mg/kg facilitated a shift to a visual cue strategy. ** = p < .01 vs. SAL – SAL. C) Mean (± SEM) perseverative errors committed in the switch to visual cue discrimination. CDD-0102A 0.03 and 0.1 mg/kg significantly decreased perseverative errors. * = p < .05 vs. SAL – SAL, ** = p < 0.01 vs. SAL – SAL. D) Mean (± SEM) regressive errors committed in the switch to visual cue discrimination. CDD-0102A 0.03 and 0.1 mg/kg significantly decreased regressive errors. ** = p < 0.01 vs. SAL – SAL. E) Mean (± SEM) never-reinforced errors committed in the switch to visual cue discrimination. CDD-0102A 0.03 and 0.1 mg/kg significantly decreased never-reinforced errors. * = p < .05 vs. SAL – SAL, ** = p < 0.01 vs. SAL – SAL.

Figure 4: The effect of CDD-0102A on visual cue acquisition and switch to a place discrimination. Each rat received an i.p. injection of saline (SAL) or one of three doses of CDD-0102A (CD) 30 minutes prior to acquisition and switch phases. The treatments in the legends represent the treatment received prior to acquisition (top row) followed by the treatment received prior to the switch phase (bottom row). A) Mean (± SEM) trials to criterion on acquisition of a visual cue discrimination. Injection of CDD-0102A had no effect on acquisition. B) Mean (± SEM) trials to criterion on switch to a place strategy. CDD-0102A at 0.03 and 0.1 mg/kg facilitated a shift to a place strategy. ** = p < .01 vs. SAL – SAL. C) Mean (± SEM) perseverative errors committed in the switch to visual cue discrimination. CDD-0102A 0.03 and 0.1 mg/kg significantly decreased perseverative errors. ** = p < 0.01 vs. SAL – SAL. D) Mean (± SEM) regressive errors committed in the switch to visual cue discrimination. CDD-0102A 0.03 and 0.1 mg/kg significantly
decreased regressive errors. ** = p < 0.01 vs. SAL – SAL. E) Mean (± SEM) never-reinforced errors committed in the switch to visual cue discrimination. CDD-0102A treatment did not affect never-reinforced errors.

Figure 5. Salivary output over a 60 min time period following i.p. administration of CDD-0102A. Data represent the mean (± SEM) from three animals for each dose.
Figure 1
Figure 2
Figure 3
Figure 4