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Intrahippocampal Norleucine[1]-Angiotensin IV mitigates scopolamine-induced spatial working memory deficits

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ABSTRACT

Depletion of cholinergic neurons in the hippocampus has been implicated in memory impairment and Alzheimer's Disease (AD). The brain angiotensin AT_4 receptor is co-localized with cholinergic neurons, and the AT_4 receptor has also been implicated in cognitive processing. The current investigation used the spatial win-shift version of the radial arm maze to determine the involvement of AT_4 receptors in spatial working memory formation. We initially established that intrahippocampal scopolamine significantly impaired the spatial working memory performance of Sprague-Dawley rats in the radial arm maze. We also demonstrated that subsequent intrahippocampal infusions of Norleucine[1]-Angiotensin IV (Nle[1]-AngIV) significantly prevented the scopolamine-induced deficit. Consistent with previously published data on long-term spatial memory, our findings suggest that activation of AT_4 receptors can compensate for impaired spatial working memory resulting from compromised muscarinic acetylcholine receptor function. We further demonstrate that the hippocampus is a site of action for Nle[1]-AngIV-mediated cognitive improvement.

1. Introduction

Alzheimer's Dementia (AD) is a progressive neurological disorder accompanied by debilitating behavioral disturbances including progressive cognitive decline. Cholinergic deficits in the forebrain are a contributing factor to the cognitive decline prevalent in AD and the majority of pharmaceutical treatments for AD target the forebrain cholinergic system [21]. In addition to its clinical relevance, the role of the cholinergic system in normal learning and memory formation is well established in the literature. Cholinergic agonists are frequently shown to improve experimentally induced memory deficits, and cholinergic antagonists reliably impair memory formation in a variety of species and tasks. Systemic and intracerebroventricular (icv) injections of scopolamine, a muscarinic acetylcholine (mACh) receptor antagonist, have long been used as an animal model of the cholinergic impairment seen in AD, but this practice has recently come under scrutiny because of concerns that scopolamine affects non-memory functions such as locomotor activity, anxiety, or sensory discrimination [13]. Microinjecting scopolamine directly into the hippocampus can overcome some of the criticisms of peripheral and icv scopolamine injection. Again, when injected directly into the hippocampus, scopolamine impairs performance on a variety of memory tasks such as inhibitory avoidance conditioning [2], novel object detection [12], and spatial memory tasks such as the water maze [10] and radial arm maze (RAM) [18,31].

The AT_4 angiotensin receptor subtype has been implicated in cognitive function [1,29]. Evidence for the involvement of AT_4 receptors in memory formation is taken from neuroanatomical, electrophysiological, and pharmacological studies. First, AT_4 receptors are localized in brain structures known to be critical for memory formation including the cerebral cortex, nucleus basalis, medial septum, and areas CA1–3 of the hippocampus [5,19,20]. Second, AT_4 receptor activation enhances long-term potentiation (LTP) in hippocampal slices [6,14,27]. Third, icv injection of AT_4 antagonists impairs memory while AT_4 agonists have been shown to improve memory [28,30]. Despite the wealth of evidence supporting the role of the AT_4 receptor in memory formation, we would be remiss if we failed to acknowledge the recent assertion that the AT_1 receptor may mediate some of the memory-enhancing properties of AT_4 agonists [7].

The idea that the AT_4 receptor system might interact with cholinergic signaling is supported by several pharmacological studies of animal behavior. AT_4 receptor agonists such as angiotensin IV (Ang IV), LVV-hemorphin-7, and the stable, high affinity Ang IV analog Norleucine[1]-Ang IV (Nle[1]-AngIV), can overcome impairments in spatial memory induced by mAChR blockade [15,26]. Further

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Nle[1]-AngIV can prevent spatial memory deficits caused by nicotinic acetylcholine receptor (nAChR) blockade [23]. However, Olson et al. showed that Nle[1]-AngIV failed to compensate for spatial memory deficits induced by both mAChR and nAChR blockade, a finding which lends support to the idea that Nle[1]-AngIV might facilitate memory by potentiating cholinergic activity [23]. In agreement with this assertion, Lee and colleagues demonstrated that Ang IV and LVV-hemorphin-7 potentiate the release of ACh in hippocampal slices, an effect that was blocked by the AT_4 receptor antagonist divalinal-AngIV [16]. A recent paper by De Bundel also reports an Ang IV-mediated modulation of hippocampal ACh levels [7], further supporting the contention that potentiation of hippocampal ACh release could be a mechanism by which Ang IV analogs facilitate cognitive performance.

The purpose of the present study was to pharmacologically test whether scopolamine-induced mAChR blockade in the dorsal hippocampus disrupted spatial working memory formation in a RAM task and whether intrahippocampal injections of Nle[1]-AngIV could overcome the scopolamine-induced deficits in spatial working memory. By microinjecting our compounds into the dorsal hippocampus we are able to determine whether the hippocampus is a site of action for Nle[1]-AngIV-mediated spatial working memory improvement. Spatial memory investigation has often involved tasks in the RAM. A variant of the delayed response task, the spatial win-shift (SWSh) task, utilizes a delay period in the RAM to test an animal's ability to remember recent spatial variables that guide decision making [22]. Importantly, the SWSh task prioritizes hippocampal functions such as working memory, spatial memory, and cognitive flexibility [4,11,24]. In addition, interference with hippocampal functioning predicts SWSh scores, whereas interference with other limbic structures does not [17]. Further, the SWSh task is sensitive enough to dissociate between delay-dependent and delay-independent spatial working memory impairments [9]. These properties make the SWSh task an effective behavioral assay for measuring spatial memory function in animal subjects.

2. Materials and methods

2.1. Animal subjects

All experimental procedures were carried out in compliance with NIH guidelines for the care and use of laboratory animals. Likewise, all procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Concordia College.

Male Sprague-Dawley rats weighing between 350 and 550 g at the start of the experiment were used in this research. Rats were housed in pairs and were kept in a room with a 12-h light/dark cycle beginning at 07:00. Ten days prior to behavioral training animals were restricted to 10–15 g food/day in order to reach a target weight (90% of their free-feed weight). This target weight was maintained throughout the experiment. Access to water was not restricted. Animals were exposed to human contact 5 min/day for 3 days prior to behavioral training.

2.2. Behavioral acquisition training

All behavioral training and testing in this investigation occurred in a RAM constructed from opaque Plexiglas. The apparatus consisted of an octagonal center platform (27.5 cm in diameter) and 8 arms (61 cm long, 12.5 cm wide, and 21 cm high) extending from each side of the center platform. The maze was elevated 0.8 m above the ground and was located in a 2×2.5 m room. Large, brightly colored shapes served as distinct extra-maze cues on 3 of the 4 walls of the room. The experimenter sat in front of the fourth wall, visible to the subjects inside of the maze. A food dish (3 cm in diameter and 1.5 cm deep) was secured to the floor at the end of each arm.

2.3. Spatial win-shift task

Prior to acquisition of the SWSh task, all experimental subjects were habituated to the maze 10 min/day for 3 days. During the habituation sessions food was scattered throughout the maze (in the center, arms, and food cups) and animals were allowed to freely explore and consume the food. After habituation, acquisition training of the SWSh task commenced.

Acquisition training trials took place over 16–19 days with each trial consisting of 2 phases. In Phase-1, ½ Honey nut Cheerio® was placed in the food cup of all 8 arms, but 4 semi-randomly selected arms (never more than two in a row) were blocked. The combination of blocked arms changed daily but all animals shared the same combination during a given day. Phase-1 ended after all 4 open arms were entered with all 4 paws or 300 s had passed. After Phase-1, animals were placed in their home cage for 5 min and then reentered the maze for Phase-2. All arms were unblocked during Phase-2, but only arms that were blocked during Phase-1 contained food. Optimal Phase-2 performance was defined as entry into only the 4 arms that contained food, without repeating any entries. Phase-2 ended when all 4 correct arms had been entered or 300 s elapsed. Errors during Phase-2 were defined as entry into any arm that did not contain food and were divided into 2 types. Across-phase errors were defined as any entry into an arm that was unblocked during Phase-1. Within-phase errors were defined as repeated entry into an arm that was blocked during Phase-1. Entry into an incorrect arm was defined as either an across- or within-phase error; multiple entries into arms that were blocked in Phase-1 were not considered within-errors. During both phases of the SWSh task the time (in seconds) to complete the phase and the order of arms entered were recorded. The rats were trained on the SWSh task until they reached baseline levels of performance (16–19 days). Baseline was defined as a group average of less than 2 across-phase errors and less than 0.3 within-phase errors for 3 consecutive days. At the end of acquisition training, rats that adopted a sequential strategy (choosing arm 8, then 7, then 6, etc.) and rats that failed to explore the RAM (fewer than 4 arm choices in 300 s) were excluded from further analysis. A total of 3 animals were excluded from our study based on these criteria.

Following task acquisition, rats were divided into 3 groups and underwent cannulation surgery. After a 5-day recovery period animals completed 3 days of post-surgical SWSh training with no injections to re-establish behavioral baseline. Following the 3 days of post-surgical baseline training, animals completed 3 days of injection training. On injection training days, the first group received an injection of artificial cerebrospinal fluid (aCSF) 20 min and 10 min prior to Phase-1 (aCSF group), the second group received an injection of scopolamine 20 min and an injection of aCSF 10 min prior to Phase-1 (Scop group), and the third group received an injection of scopolamine 20 min prior and an injection of Nle[1]-AngIV 10 min prior to Phase-1 (Scop/Nle[1]-AngIV group). Given logistical constraints, 3 sets of rats were trained and injected over the course of 1 year. The first set contained only animals from the aCSF and Scop groups. The second and third sets of rats included animals from the aCSF, Scop, and Scop/Nle[1]-AngIV groups. Clearly, training animals in 3 separate groups can lead to the introduction of extraneous variables; however, dependent measures for each group were statistically similar across the 3 sets of animals.

2.4. Surgery

All animal subjects were injected with equithesin (3 ml/kg, i.p.; active ingredient sodium pentobarbital 30 mg/kg; Sigma P-