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Central administration of angiotensin IV rapidly enhances novel object recognition among mice

Jason J. Paris, Shainnel O. Eans, Elisa Mizrachi, Kate J. Reilley, Michelle L. Ganno, Jay P. McLaughlin*

Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port Saint Lucie, FL 34987, USA

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ABSTRACT

Angiotensin IV (Val^1-Tyr^2-Ile^3-His^4-Pro^5-Phe^6) has demonstrated potential cognitive-enhancing effects. The present investigation assessed and characterized: (1) dose-dependency of angiotensin IV's cognitive enhancement in a C57BL/6J mouse model of novel object recognition, (2) the time-course for these effects, (3) the identity of residues in the hexapeptide important to these effects and (4) the necessity of actions at angiotensin IV receptors for procognitive activity. Assessment of C57BL/6J mice in a novel object recognition task demonstrated that prior administration of angiotensin IV (0.1, 1.0, or 10.0, but not 0.01 nmol, i.c.v.) significantly enhanced novel object recognition in a dose-dependent manner. These effects were time dependent, with improved novel object recognition observed when angiotensin IV (0.1 nmol, i.c.v.) was administered 10 or 20, but not 30 min prior to the onset of the novel object recognition testing. An alanine scan of the angiotensin IV peptide revealed that replacement of the Val^1, Ile^3, His^4, or Phe^6 residues with Ala attenuated peptide-induced improvements in novel object recognition, whereas Tyr^2 or Pro^5 replacement did not significantly affect performance. Administration of the angiotensin IV receptor antagonist, divalinal-Ang IV (20 nmol, i.c.v.), reduced (but did not abolish) novel object recognition; however, this antagonist completely blocked the procognitive effects of angiotensin IV (0.1 nmol, i.c.v.) in this task. Rotorod testing demonstrated no locomotor effects with any angiotensin IV or divalinal-Ang IV dose tested. These data demonstrate that angiotensin IV produces a rapid enhancement of associative learning and memory performance in a mouse model that was dependent on the angiotensin IV receptor.

1. Introduction

The increased prevalence of neurological disorders (Brookmeyer et al., 2007; Small et al., 1997) highlights the urgent need for new drugs to treat cognitive decline. Although research has focused on known deficiencies associated with Alzheimer's disease, clinical studies of cognitive enhancement with cholinesterase inhibitors (Birks, 2006; Raina et al., 2008) or antioxidants (Nunomura et al., 2007) have been disappointing, prompting the search for new pharmacological options.

Initially identified as a potent vasoconstrictor (De Bundel et al., 2008; Wright et al., 1993; Yang et al., 2008), intracerebroventricular (i.c.v.) administration of angiotensin II (Ang II) has been found to enhance the cognitive performance of rats, facilitating acquisition of conditioned avoidance (Baranowska et al., 1983; Braszko, 2002) and delayed object recognition (Braszko, 1996). These effects were independent of actions at Ang II's native receptor (AT_2) or AT_1 receptors (Baranowska et al., 1983; Braszko, 2002), and were attributed to actions of natural Ang II metabolites, including the hexapeptide, angiotensin IV (Val^1-Tyr^2-Ile^3-His^4-Pro^5-Phe^6; Ang IV). In fact, Ang IV may possess the greater therapeutic potential as Ang IV is observed to bind with high affinity in sites associated with mnemonic performance, including the pyramidal layer of the hippocampus (CA1, CA2, CA3), dentate gyrus, basal nucleus of Meynert, and throughout the neocortex of guinea pigs (Harding et al., 1992; Miller-Wing et al., 1993; Wright et al., 1993), rats (Roberts et al., 1995), and humans (Chai et al., 2000). Consistent with this, central administration of Ang IV induced cognitive improvements equivalent to Ang II in rats (Braszko et al., 1988), and the blockade of AT_4 receptors produced deficits in spatial learning (Wilson et al., 2009; Wright et al., 1999). Agonism of AT_4 receptors has been reported to promote hippocampal long-term potentiation (LTP;

Abbreviations: Ang, angiotensin; AT, angiotensin receptor; IRAP, insulin-responsive aminopeptidase; i.c.v., intracerebroventricular; LTD, long-term depression; LTP, long-term potentiation; SEM, standard error of the mean.

* Corresponding author. Tel.: +1 772 345 4715; fax: +1 772 345 3649.
E-mail addresses: jparis@tpims.org (J.J. Paris), shaieans@hotmail.com (S.O. Eans), mizzelisa02@gmail.com (E. Mizrachi), katejreilley@gmail.com (K.J. Reilley), condor1082@aol.com (M.L. Ganno), jmclaughlin@tpims.org (J.P. McLaughlin).

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Kramár et al., 2001; Wayner et al., 2001), spatial learning (Lee et al., 2004; Wright et al., 1999), and the restoration of spatial cognitive function following a variety of central insults (Stubley-Weatherly et al., 1996; Wright et al., 1996, 1999; Pederson et al., 2001).

In the present investigation, we assessed the dose–response relationship of Ang IV-enhanced learning and memory using a truncated version of the novel object recognition task. We have previously demonstrated that shortened paradigm parameters reduce the ability of control C57BL/6J mice to recognize a novel object (Carey et al., 2009). We hypothesized that central administration of Ang IV would enhance novel object recognition in a dose- and time-dependent manner under these conditions, independent of effects on locomotion, and that pharmacological blockade of the AT_4 receptor would attenuate these enhancements. Moreover, although others have demonstrated that the valine[1] residue in Ang IV is essential for function of the peptide, whereas the proline[5] and phenylalanine[6] residues are not (Benoist et al., 2011; De Bundel et al., 2008; Sardinia et al., 1994), we conducted a complete alanine scan of the hexapeptide to assess the necessity of each residue for Ang IV-mediated enhancement of cognition.

2. Materials and methods

2.1. Animals, housing and animal assurance

Subjects were 390 adult (approximately 70 days of age), male C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in a temperature- and humidity-controlled room at the Torrey Pines Institute for Molecular Studies (Port Saint Lucie, FL) vivarium on a 12:12 h light/dark cycle (lights off at 19:00 h) with *ad libitum* access to food and water.

All animal experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Eighth edition, revised 2011). Consistent with these guidelines, ongoing statistical testing of data collected was used to minimize the number of animals used, within the constraints of necessary statistical power. All methods used were pre-approved by the Institutional Animal Care and Use Committee at the Torrey Pines Institute for Molecular Studies (Port Saint Lucie, FL).

2.2. Intracerebroventricular administration technique

Per the modified method of Haley and McCormick (1957), i.c.v. injections were directly made into the lateral ventricle as described by our group and others (Toll et al., 2012; Van Heek et al., 1997; Zadina et al., 1997; Zhu et al., 1999). Briefly, mice were lightly anesthetized with isoflurane, a small incision was made in the scalp, and an injection was made 2 mm lateral and 2 mm caudal to bregma, at a depth of 3 mm. The volume of all i.c.v. injections was 5 μl, using a 10 μl syringe (Hamilton Co., Reno, NV, USA). The syringe was left in place for 5 s to allow for diffusion of the infusate. Note that mice do not require a suture following i.c.v. drug administration and the quick (<1 min) recovery time from anesthesia occurs within the drug pretreatment times. We have previously compared this i.c.v. drug administration method to intraperitoneal administration and have found no impairment of novel object recognition (Paris et al., 2011).

2.3. Peptide synthesis

Angiotensin IV (VYIHPF), and Ang IV variants with alanine substitution at each position (V1A, Y2A, I3A, H4A, P5A, and F6A) were obtained from GenScript (Piscataway, NJ, USA). The putative AT_4 antagonist, divalinal-Ang IV [Vψ(CH_2-NH_2) YVψ(CH_2-NH_2)HPF; Krebs et al., 1996] was obtained from AnaSpec (San Jose, CA, USA). Synthetic peptides were diluted to concentration in 0.9% sterile saline.

2.4. Behavioral assays

2.4.1. Rotorod testing

In Experiment 1, locomotion was assessed in the rotorod task, which was conducted as previously described (Paris et al., 2011). Briefly, mice were trained to balance on an immobile rotorod (3 cm in diameter and suspended approximately 46 cm high; San Diego Instruments, San Diego, CA, USA) for 30 s. Mice were then assessed on the rotating rotorod across three fixed speed trials (30 s max. latency at 10 rpm), two fixed speed trials (180 s max. latency at 10 rpm), and two accelerated speed trials (180 s max. latency at 0–20 rpm). After each trial, the latency to fall from the rotorod was recorded. The last of these trials was utilized as a baseline measure of rotorod performance. One hour later, mice were administered drug and were assessed in accelerated speed trials (180 s max. latency at 0–20 rpm) over a 30 min period. Increased latencies to fall indicate increased motor performance.

2.4.2. Novel object recognition

Mice were assessed in a novel object recognition paradigm as previously described (Carey et al., 2009, 2012; Paris et al., 2011). Novel object recognition is a cognitive behavioral assay that is dependent on the activity of the frontal cortex and hippocampus (Broadbent et al., 2004; Ennaceur et al., 1997). The testing paradigm consists of 3 phases (two acquisition trials followed by one retention trial; each separated by an inter-trial interval; Carey et al., 2009, 2012; Paris et al., 2011). The duration of the phases and inter-trial intervals influence performance, as detailed below. In general, in the first phase, mice explore two objects (cube playing dice; 16 × 16 × 16 mm) that are evenly placed at opposing ends of a clean mouse housing cage (16 × 24 × 12 cm). Object exploration was operationally defined as the duration of time mice spent physically contacting the object with a part of the body other than the tail and when mice were facing the object (within 0.5 cm of the object) and actively exploring it (via sniffing or physical manipulation) as previously described by Carey et al. (2009). In Phase II, one of the familiar objects is displaced (moved from a centralized position at the end of the cage into close proximity to the wall). In the last phase, one of the familiar objects is replaced with a novel object (a marble; 1.25 cm diameter).

The constant object in this study was always a centralized playing die and the target object in Phase III was a marble. Previous work has shown that neither playing dice nor marbles present as a more salient cue to C57BL/6J mice, and that these objects are inter-changeable in this protocol, with no significant difference in performance when a playing die or marble was used as the novel object ($F(1,33) = 0.316$; $p = 0.578$, one-way ANOVA), and no significant interaction of stimuli × phase ($F(2,100) = 0.96$; $p = 0.39$, two-way ANOVA) as previously reported in Carey et al. (2009).

In each phase, the amount of time mice spent exploring each object was recorded. Time spent exploring each object was calculated as a recognition index: [time spent investigating target object/(time spent investigating constant object + time spent investigating target object) × 100]. A significant increase in percentage of time spent with the novel object in Phase III is considered an index of enhanced learning and memory performance (Carey et al., 2009, 2012; Paris et al., 2011).

2.4.2.1. Standard novel object recognition (10 min trials). In Experiment 3A, mice were assessed in a standard length novel object recognition paradigm, where the phases of the testing paradigm are each 10 min long, each separated by a 10 min inter-trial interval (Carey et al., 2012; Paris et al., 2011). In control C57BL/6J subjects, these timing parameters typically yield a significant increase from ~50% exploration of the target object in Phases I and II to ~70–80% exploration with the target novel object in Phase III (Carey et al., 2009, 2012; Paris et al., 2011), indicative of positive learning and memory performance. Only Experiment 3A, which evaluated possible AT_4 receptor antagonist-mediated impairment of novel object recognition, utilized the standard task parameters (10 min trials with 10 min inter-trial intervals).

2.4.2.2. Truncated novel object recognition (5 min trials). In Experiments 2–4 (excluding Expt. 3A) mice were assessed in a novel object recognition paradigm utilizing truncated parameters. The truncated novel object recognition paradigm employs shorter (5 min) acquisition and retention trials and shorter (1 min) inter-trial intervals, presumably weakening the consolidation of the memory trace for the novel object (Genoux et al., 2002). As previously reported, utilization of these parameters resulted in an insignificant difference in the amount of time that control mice spent investigating the novel object across phases (Carey et al., 2009), suggestive of negative learning and memory performance. Given the goals of the present study, we utilized this truncated version of the novel object recognition task in Experiments 2, 3B and 4 to evaluate Ang IV-mediated enhancement of learning and memory performance.

2.5. Experimental schedule and drug dosing

2.5.1. Experiment 1: locomotor effects of central angiotensin IV or divalinal-Ang IV

Locomotor behavior was assessed via performance in the rotorod task. Following rotorod training (described in detail above), mice were administered central vehicle (sterile saline, 0.9%, i.c.v.; $n = 16$), Ang IV (0.01, 0.1, 1.0, or 10 nmol, i.c.v.; $n = 8$/group), or divalinal-Ang IV (20 nmol, i.c.v.; $n = 8$). Mice were assessed for rotorod performance at 0, 10, 20, and 30 min, post-administration.

2.5.2. Experiment 2: dose- and time-dependent effects of central angiotensin IV on novel object recognition

To assess dose-dependent effects of Ang IV on novel object recognition, prior to Phase I of the task (described in detail above), mice were unilaterally administered (i.c.v.) sterile saline (0.9%; $n = 15$), or one of four doses of Ang IV (0.01 nmol, $n = 17$; 0.1 nmol, $n = 20$; 1.0 nmol, $n = 23$; 10 nmol, $n = 20$). Mice began testing in Phase I of the task 10 min later.

To assess the duration of Ang IV action for effects on novel object recognition, the pretreatment time between drug administration and testing in Phase I of the