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Angiotensin IV and LVV-haemorphin 7 enhance spatial working memory in rats: Effects on hippocampal glucose levels and blood flow

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ABSTRACT

The IRAP ligands Angiotensin IV (Ang IV) and LVV-haemorphin 7 (LVV-H7) enhance performance in a range of memory paradigms in normal rats and ameliorate memory deficits in rat models for amnesia. The mechanism by which these peptides facilitate memory remains to be elucidated. In recent *in vitro* experiments, we demonstrated that Ang IV and LVV-H7 potentiate activity-evoked glucose uptake into hippocampal neurons. This raises the possibility that IRAP ligands may facilitate memory in hippocampus-dependent tasks through enhancement of hippocampal glucose uptake. Acute intracerebroventricular (i.c.v.) administration of 1 nmol Ang IV or 0.1 nmol LVV-H7 in 3 months-old Sprague-Dawley rats enhanced spatial working memory in the plus maze spontaneous alternation task. Extracellular hippocampal glucose levels were monitored before, during and after behavioral testing using *in vivo* microdialysis. Extracellular hippocampal glucose levels decreased significantly to about 70% of baseline when the animals explored the plus maze, but remained constant when the animals were placed into a novel control chamber. Ang IV and LVV-H7 did not significantly alter hippocampal glucose levels compared to control animals in the plus maze or control chamber. Both peptides had no effect on hippocampal blood flow as determined by laser Doppler flowmetry, excluding that either peptide increased the hippocampal supply of glucose. We demonstrated for the first time that Ang IV and LVV-H7 enhance spatial working memory in the plus maze spontaneous alternation task but no *in vivo* evidence was found for enhanced hippocampal glucose uptake or blood flow.

1. Introduction

Insulin-regulated aminopeptidase (IRAP) was initially characterized in adipocytes as a marker protein of specialized vesicles containing the insulin-regulated glucose transporter-4 (GLUT4) (Keller, Scott, Mastick, Aebersold, & Lienhard, 1995; Ross et al., 1996). IRAP is a membrane bound aminopeptidase with a large extracellular catalytic domain, a single transmembrane domain and an extended cytoplasmic domain containing two dileucine motifs preceded by acidic clusters which are associated with trafficking (Johnson, Lampson, & McGraw, 2001; Keller et al., 1995). In adipocytes, IRAP redistributes with GLUT4 to the plasma membrane in response to insulin. IRAP is also found in other tissues such as the brain (Keller et al., 1995), where it is predominantly expressed in neurons, with high concentrations in areas involved in cognitive functions such as the hippocampus, the amygdala, the basal forebrain and the cerebral cortex (Fernando, Larm, Albiston, & Chai, 2005). Intriguingly, in these brain areas, IRAP and GLUT4 are co-localized in secretory vesicles, similarly to the insulin-regulated vesicles of adipocytes, suggesting that IRAP may be involved in the regulation of GLUT4 dependent neuronal glucose uptake (Fernando, Luff, Albiston, & Chai, 2007).

The role of IRAP in the brain remained elusive until it was identified as a high affinity binding site for two endogenous peptides, angiotensin IV (Ang IV) and LVV-haemorphin 7 (LVV-H7) (Albiston et al., 2001). Acute or chronic administration of these IRAP ligands into the cerebral ventricles of rats enhances performance in a range of memory paradigms including the passive avoidance (Braszko, Kupryszewski, Witczuk, & Wisniewski, 1988; Wright et al., 1993), Barnes circular maze (Lee et al., 2004) and Morris water maze (Wright et al., 1999) tasks. Moreover, IRAP ligands ameliorate memory deficits induced by chronic alcohol exposure (Wisniewski, Borawska, & Car, 1993), global ischemia (Wright et al., 1996), bilateral perforant pathway lesion (Wright et al., 1999), or chemical perturbations of the septo-hippocampal cholinergic pathways (Albiston et al., 2004; Olson et al., 2004; Pederson, Krishnan, Harding, & Wright, 2001).

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Whereas several brain areas may be involved in the different memory effects of IRAP ligands, many studies have focused on the hippocampus because of the robust effects of these peptides on the hippocampal-dependent spatial learning. The observation that i.c.v. administration of Ang IV predominantly evokes c-FOS expression in the hippocampus (Roberts et al., 1995) indeed suggests that this brain area is a major target site for the memory effects of IRAP ligands. In support of their spatial memory enhancing effects, IRAP ligands were demonstrated to facilitate long-term potentiation in the CA1 region of the hippocampus *in vitro* (Kramar et al., 2001) and in the dentate gyrus *in vivo* (Wayner, Armstrong, Phelix, Wright, & Harding, 2001). Furthermore, Ang IV and LVV-H7 facilitated potassium-evoked acetylcholine release in rat hippocampal slices (Lee, Chai, Mendelsohn, Morris, & Allen, 2001).

The ability of IRAP ligands to facilitate memory in both normal animals and a range of memory deficit models makes IRAP a potential therapeutic target. However, the mechanism by which IRAP ligands facilitate memory is not fully understood. Several candidate mechanisms have been proposed including facilitation of neuronal glucose uptake (Albiston et al., 2003; De Bundel, Smolders, Vanderheyden, & Michotte, 2008). In recent *in vitro* experiments, we demonstrated that Ang IV and LVV-H7 potentiate activity-evoked glucose uptake into mice hippocampal neurons (Fernando, Albiston, & Chai, 2008). This effect was absent in IRAP knockout mice, strongly suggesting a role for IRAP in hippocampal glucose uptake (Fernando et al., 2008). IRAP ligands may therefore enhance spatial memory through potentiation of glucose uptake into hippocampal neurons. Indeed, it is well established that glucose is a potent modulator of memory. Systemic or intrahippocampal glucose administration facilitates spatial memory in rats (Dash, Orsi, & Moore, 2006; McNay & Gold, 1999). Furthermore, a previous microdialysis study elegantly demonstrated a decrease in extracellular glucose levels in the hippocampus of rats performing a plus maze spontaneous alternation task (McNay, Fries, & Gold, 2000). This decrease in extracellular glucose is indicative of enhanced glucose uptake by hippocampal cells during spatial memory processing. We hypothesized that under these conditions, Ang IV and LVV-H7 may facilitate hippocampal glucose uptake similarly to our observations *in vitro*.

The present study uses the *in vivo* microdialysis technique to test this hypothesis. We investigated the effects of Ang IV and LVV-H7 on hippocampal glucose levels during performance of a spatial working memory task. Since the hippocampal extracellular glucose concentration not only depends on the local usage but also on the supply from the blood, we further determined the effects of Ang IV and LVV-H7 on hippocampal blood flow. Identification of the mechanism by which IRAP ligands facilitate memory may provide a novel strategy for the treatment of cognitive impairment.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (Animal Resource Centre, Canning Vale, WA, Australia and Charles-River Laboratories, L'Arbresle, Cedex, France), 3 months old at the time of surgery, were housed individually in an animal housing facility maintained at a 12/12 h light/dark cycle initiated at 7:00 h. All animals were given free access to water and standard rat chow. The experiments were carried out in accordance with the Prevention of Cruelty to Animals Act and the National Health and Medical Research Council Code of Practice for the Use of Animals for Scientific Purposes in Australia and according to the European directive 86/609/EEC with the corresponding national guidelines as approved by the Ethical Committee for Animal Experimentation of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel.

2.2. Surgery

2.2.1. Behavior and microdialysis experiments

Rats were anesthetized with 5% (v/v) isoflurane (Fluothane; ICI, Melbourne, Australia), placed on a stereotaxic frame and maintained on 2% (v/v). Rectal temperature was maintained at 37.5 °C using a heating pad. The rats were stereotaxically implanted with a microdialysis guide cannula (CMA/12; CMA Microdialysis, Solna, Sweden) above the left or right hippocampus and an injection guide cannula (22 g; Plastics One, Roanoke, USA) above the ipsilateral ventricle. The location of both the microdialysis guide cannula and injection guide cannula were randomized between left and right hemisphere for different animals within each experimental group. The flat skull coordinates were 4.6 mm lateral from midline, 5.6 mm posterior from bregma and 3.6 mm ventral from dura for the microdialysis guide cannula aimed at the hippocampus and 1.5 mm lateral from midline, 0.8 mm posterior to bregma and 3.5 mm ventral from dura for the injection guide cannula aimed at the lateral ventricle. After stereotaxic positioning, the guide cannulae were secured to the skull with stainless steel screws and dental cement. Rats received an intramuscular injection of 0.05 mg/kg buprenorphine (Temgesic; Reckitt Benckiser, West Ryde, Australia) and a subcutaneous injection of 10 mg/kg enrofloxacin (Baytril; Bayer, Pymble, Australia). Dummy cannulae were introduced into the guide cannulae to prevent occlusion. Rats were allowed to recover for a minimum of 6 days and were handled daily for at least 1 min during this time. On completion of testing, the rats received an overdose of Lethobarb (Virbac, Peakhurst, Australia) and the brains were removed for verification of the guide cannula localizations.

2.2.2. Hippocampal blood flow experiments

Rats were anesthetized with an intraperitoneal injection of 60 mg/kg pentobarbital (Nembutal; Ceva Sante Animal, Brussels, Belgium) and maintained with an intraperitoneal infusion of 0.06 mg/kg pentobarbital at a flow rate of 1 μl/min. Tracheal intubation was performed to facilitate breathing throughout the experiment and the right jugular vein of some of the rats was cannulated for supplementary drug administration. The animals were placed on a stereotaxic frame and rectal temperature was maintained at 37.5 °C using a heating pad. The rats were stereotaxically implanted with an injection guide cannula (22 g; Plastics One, Roanoke, USA) above the left or right ventricle and a circular craniotomy with 2 mm diameter was performed above the ipsilateral hippocampus. The location of both the injection guide cannula and the craniotomy were randomized between left and right hemisphere for different animals within each experimental group. The flat skull coordinates were 1.5 mm lateral from midline, 0.8 mm posterior to bregma and 3.5 mm ventral from dura for the injection guide cannula aimed at the lateral ventricle. The injection guide cannula was secured to the scull with a screw and dental cement. The craniotomy above the hippocampus was performed at flat skull coordinates 4.6 mm lateral from midline and 5.6 mm posterior from bregma. After careful removal of the dura, a laser Doppler recording probe (0.8 mm diameter; Vasamed, Eden Prairie, USA) was lowered 3.6 mm ventral from dura to measure blood flow in the hippocampus. On completion of testing, the rats received an overdose of pentobarbital and the brains were removed for verification of the guide cannula and laser Doppler recording probe localizations.

2.3. Peptide administration

In all experiments, the peptides were administered into the lateral ventricle with a 10 μl Hamilton syringe (Scientific Glass Engineering, Ringwood, Australia) via a FEP tubing (CMA Microdialysis, Sweden), which was connected to the injection needle (30 g; Plastics One, Roanoke, USA). The injection needle protruded