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Forebrain neurone-specific deletion of insulin-regulated aminopeptidase causes age related deficits in memory

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ABSTRACT

Central infusion of Insulin-Regulated Aminopeptidase (IRAP) inhibitors improves memory in both normal rodents and in models of memory deficit. However, in contrast, the global IRAP knockout mice (KO) demonstrate age-accelerated spatial memory deficits and no improvements in performance in any memory tasks. Potentially, the observed memory deficit could be due to the absence of IRAP in the developing brain. We therefore generated a postnatal forebrain neuron-specific IRAP knockout mouse line (CamKIIalphaCre; IRAPlox/lox). Unexpectedly, we demonstrated that postnatal deletion of IRAP in the brain results in significant deficits in both spatial reference and object recognition memory at three months of age, although spatial working memory remained intact. These results indicate a significant role for IRAP in postnatal brain development and normal function of the hippocampus in adulthood.

1. Introduction

Insulin-regulated aminopeptidase (IRAP) is the AT_4 receptor, the binding site for the AT_4 ligands angiotensin IV (Ang IV, VYIHPF) and LVV-hemorphin 7 (LVV-H7, LVVYPWTQRF) (Albiston et al., 2001). Central infusion of AT_4 ligands improves memory in both normal rodents (Lee et al., 2004; Wright et al., 1999) and in models of memory deficit (Albiston et al., 2004; Wright et al., 1999). Other binding sites have been proposed to mediate the cognitive effects of Ang IV and its analogues (Wright & Harding, 2011), but we recently demonstrated the complete absence of Ang IV binding in IRAP knockout mouse tissues (Albiston et al., 2010), confirming IRAP as the primary site of action. The prevalence of IRAP in hippocampal and cortical excitatory neurons supports the hypothesis that IRAP plays an important role in learning and memory (Fernando, Larm, Albiston, & Chai, 2005), but the precise mechanisms of action remain to be identified.

The effects of Ang IV and other AT_4 ligands have been attributed to their inhibition of IRAP's catalytic activity, and the consequential increased half-life in endogenous peptide substrates (Albiston et al., 2008; Stragier et al., 2008). IRAP cleaves a variety of neuropeptides with potential roles in cognition; these include arginine vasopressin, oxytocin, somatostatin and met-enkephalin (Lew et al., 2003; Matsumoto et al., 2001), with IRAP knockout mice demonstrated to have increases in circulating and central vasopressin levels (Wallis, Lankford, & Keller, 2007). However, other potential mechanisms have been identified based on alternative functions of IRAP. The intracellular domain of IRAP plays a role in regulating GLUT4 vesicle trafficking within fat and muscle tissue (Larance et al., 2005; Peck et al., 2006). Interestingly, we identified an analogous association between IRAP and GLUT4 in the neurosecretory vesicles of hippocampal and cortical neurons (Fernando, Albiston, & Chai, 2008; Fernando, Luff, Albiston, & Chai, 2007), suggesting IRAP might regulate glucose uptake in the brain. Indeed, a range of AT_4 ligands enhance depolarization induced glucose uptake in mouse hippocampal slices in an IRAP and GLUT4-dependent manner (Albiston et al., 2008; Fernando et al., 2008).

We previously analysed a global IRAP null ($IRAP^{-/-}$) mouse and characterised its performance in several memory paradigms (Albiston et al., 2010). In contrast to the favourable effects of acute IRAP inhibition, $IRAP^{-/-}$ mice display an age-related spatial memory impairment from six months of age (Albiston et al., 2010). In the embryonic mouse brain there is pronounced IRAP expression in the neurogenic subventricular zone (Chai et al., 2001), suggesting that these deficits may be due to changes in brain development (Albiston et al., 2011). In an attempt to delineate the role of IRAP

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in brain development and normal function, we developed a forebrain neuron specific IRAP knockout mouse. This was achieved by crossing IRAP lox mice (Albiston et al., 2010) with mice expressing Cre under the control of the calcium calmodulin kinase IIα promoter (Casanova et al., 2001), a transgenic combination resulting in the postnatal deletion of the IRAP gene from neurons in the forebrain. We describe the behavioural phenotype of these animals, and reveal deficits in hippocampus-dependent tasks including visual recognition and spatial reference memory.

2. Methods

2.1. Animals

All experiments conducted on animals in this study were approved by the Florey Neuroscience Institutes animal ethics committee and are in accordance with the National Health and Medical Research Council of Australia guidelines. The generation of the floxed IRAP ($IRAP^{fl/fl}$) mouse has been reported previously (Albiston et al., 2010). Female $IRAP^{fl/fl}$ mice were crossed with CamKIIα iCre stud males (obtained from Dr. Günther Schütz, German Cancer Research Centre). The F2 and all subsequent generations consisted of $IRAP^{fl/fl}$ or $IRAP^{fl/fl;CamkII\alpha Cre}$ genotypes, from hereon referred to as wild type (WT) and forebrain neuron-specific IRAP knockout (fbn-IRAP KO) respectively. Genomic DNA for genotyping was extracted from tail snips with the REDExtractNAmp™ Tissue PCR kit (Sigma-Aldrich, Castle Hill, Australia). Oligonucleotide triplets for genotyping were GATAAGATAGAAGTAGGGGAGA, CAATAGAGGTACAGTCACCA and GGAGAATAAGGGCTGTGAGAGA (Genbank ID: NT_039649.8) for IRAP, and CCTGTTGTTCAGCTTGCA CCAG, CTGCATGCACGGGACAGCTCT and GGTTCTCCGTTTGCACTC AGGA (Casanova et al., 2001) for the CamKIIαCre transgene.

2.2. Western blotting

The hippocampus, cerebral cortex and cerebellum were dissected and snap frozen for preparation of membrane extracts and blotting for IRAP as described (Albiston et al., 2010).

2.3. In vitro autoradiography for detection of IRAP

Brains were dissected from mice that were decapitated following Isoflo (Fluothane_, ICI, Melbourne, Australia) anaesthesia and snap frozen by immersion in isopentane at −40 °C. Ten micron frozen coronal sections were cut, thaw mounted onto 0.5% gelatine coated slides, dried and processed for in vitro autoradiography as described previously (Albiston et al., 2010).

2.4. Behaviour

Mice were housed individually with *ad libitum* access to food and water, under a 12-h light cycle beginning at 7 am. Animals were tested at 3, 9 and 15 months of age, with individual tasks separated by at least 24 h. The body weight, food and water intake of individual mice were assessed for five consecutive days prior to behavioural assessment. Unless otherwise stated all the behavioural tests were conducted in a dimly lit (<50 lx), quiet room where the mice were habituated for a period of at least 30 min prior to testing.

2.5. Locomotor activity

The animals' locomotor activity was evaluated using an automated locomotor cell system (Coulbourn Instruments, Whitehall, USA) as described previously (Albiston et al., 2010).

2.6. Anxiety

State dependent anxiety was assessed using the elevated plus maze (EPM) as previously described (Albiston et al., 2010).

2.7. Motor coordination and balance

Motor coordination and balance were measured using a three trial accelerating rotarod paradigm as described (Albiston et al., 2010).

2.8. Object recognition memory

Visual recognition memory was assessed using an object recognition paradigm as described (Albiston et al., 2010), with some modifications. Within each cohort, mice were assigned their own arena, a 30 × 30 × 30 cm storage crate, which was not cleaned between trials (Ennaceur & Delacour, 1988). In addition, those mice not exploring the objects for more than 10 s in Trial 1, or 5 s in Trial 2, were excluded from further analyses.

2.9. Spatial reference memory

The two trial Y-maze paradigm was employed as described (Albiston et al., 2010), except that a 30-min inter-trial interval (ITI) was employed. Arm entry frequency and duration were calculated in Ethovision© XT 4.0, and the novel arm was compared to the familiar (non-start) arm for each mouse to create a frequency preference (FP) and a time preference (TP) (Wright & Conrad, 2005).

2.10. Spatial working memory

A continuous Y-maze paradigm was used to measure spontaneous alternation. The Y-maze was prepared as for trial 2 of the reference memory paradigm, and mouse behaviour was analysed as described (Senechal, Kelly, Cryan, Natt, & Dev, 2007).

2.11. Aversive conditioning

The conditioned taste avoidance (CTA) paradigm was employed as described (Pistell, Zhu, & Ingram, 2008), except that water was available between 9 am and 4 pm, and the unconditioned stimulus was 0.25 M LiCl (sodium salt, Sigma-Aldrich). Extinction was assessed over six days.

2.12. Statistical analyses

All data are presented as mean ± standard error of the mean. Student's t and ANOVA tests were performed in PASW Statistics v18.0 (SPSS Inc, Chicago, USA), with Bonferroni's post hoc for multiple comparisons. Greenhouse-Geisser ε-values were used to correct for violations of the sphericity assumption in ANOVA. Differences between groups were considered to be statistically significant if $P < 0.05$.

3. Results

The reduction in IRAP expression in hippocampus and cortex of the adult fbn-IRAP KO mouse was confirmed by western blot analysis (Fig. 1). No differences in body weight, food intake or water consumption were detected between genotypes across all ages tested (Fig. 2).