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Research report

Hippocampal protein kinase C family members in spatial memory retrieval in the mouse

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HIGHLIGHTS

- We propose a unique role for hippocampal PKC gamma in spatial memory retrieval in the CD1 mouse.
- Other PKC family members are not linked to spatial memory retrieval.
- Cave: PKC isoforms may present with more than one band on immunoblotting in the hippocampus.

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ABSTRACT

Although a few individual members of the protein kinase C (PKC) family were studied in spatial memory no systematic approach was carried out to concomitantly determine all described PKC family members in spatial memory of the mouse. It was therefore the aim of the current study to link hippocampal PKCs to memory retrieval in the Morris water maze (MWM).

CD1 mice were trained ($n = 9$) or untrained ($n = 9$) in the MWM, hippocampi were taken 6 h following the test for memory retrieval and PKCs were determined in mouse hippocampi by immunoblotting. The trained animals learned the spatial memory task and kept memory at the probe trial. PKCs alpha and epsilon were comparable between groups while PKCs beta, delta, gamma (two forms, i.e. two bands on Western blotting), zeta (2 forms) were higher in trained mice and theta (2 forms) were lower in trained mice. PKC gamma (1 form) was significantly correlating with the time spent in the target quadrant ($r = 0.7933$; $P = 0.0188$). Changes of hippocampal levels of PKCs beta, delta, gamma, zeta and theta were paralleling memory retrieval of the MWM task but correlations revealed that spatial memory retrieval was only linked to one form of PKC gamma. Results are also in agreement with a recent publication showing that PKM zeta is not required for memory formation. These findings may be relevant for the interpretation of previous work and the design of future work on the protein kinase C family in spatial memory of the mouse.

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1. Introduction

Although the involvement of several protein kinase C (PKC) isoforms for spatial memory processes has been proposed, information on the role of individual PKC family members in spatial memory mechanisms is still incomplete. Van der Zee et al. have shown that in a hole board test the trained group revealed strong PKC gamma-immunoreactivity in somata and dendrites [1]. Abeliovich et al. demonstrated that PKC gamma deficient mice exhibited mild spatial and cognitive impairment [2] and microinfusion of PKC gamma antisense oligodeoxynucleotide into the nucleus accumbens lead to impaired spatial learning in its early phase of acquisition [3]. Colombo et al. have added information to the subject that protein kinase C isoforms in young and aged rats are linked to spatial memory depending on the subcellular fractions [4]. Nogues et al. proposed that PKC activity is essentially involved in the associative component of the eight-arm radial arm maze task and it was reported that a specific inhibitor of PKC activity, polymyxin B, delayed the acquisition of the reference memory in the radial arm maze whereas long-term retention was improved

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[5]. Administration of a PKC activator at the end of the acquisition phase, however, improved long-term retention [6]. Vazquez and co-worker determined hippocampal PKA and PKC activity using a hole board spatial discrimination task and observed maximal activation at day 3 of spatial acquisition while PKA maximal activity was found on day 1 thus revealing different protein kinase profiles in spatial discrimination learning [7]. Knafo et al. observed that PKC activated LTP and spatial memory cognitive enhancement followed by calcium–calmodulin kinase II and subsequent synaptic delivery of AMPA receptors [8]. Bonini et al. have used a PKC and PKCmu inhibitor, 12-(2-cyanoethyl)-6,6,12,13-tetrahydro-13-methyl-oxo-5H-indolo[2,3-a]pyrrolo[3,4-carbazole], showing that activity of these two kinases is required in the CA1 region of rat dorsal hippocampus for acquisition and consolidation of spatial memory in the Morris water maze learning task [9]. The authors also propose that the inhibitor induces a long-lasting amnesia suggesting that post-retrieval activation of hippocampal PKC is essential for persistence of spatial memory. Evidence for the importance of PKC epsilon and alpha – activation was generated by Hongpaisan et al.: administration of the PKC epsilon and alpha activator bryostatin or PKC epsilon activator DCP-LA along with memory training restored mushroom spine synapses and enhanced spatial memory in the aged rat [10]. Sacktor reviewed work on a brain-specific isoform of PKC, PKM zeta, addressing its pivotal role for memory storage and states that PKM zeta inhibition in the hippocampus after learning eliminates retention of spatial memory [11]. Serrano et al. used two inhibitors of PKM zeta, chelerythrine and a zeta inhibitory peptide and observed disrupted retention of information for spatial reference but not working memory in the radial arm maze and precise spatial information in a water maze [12]. Volk et al., however, demonstrated, that PKM zeta is not required for hippocampal synaptic plasticity, learning and memory: the authors generated transgenic mice lacking PKC-zeta and PKM zeta and found that both, conventional and conditional knockout mice, show normal synaptic transmission and LTP at Schaffer collateral-CA1 synapses and have no deficits in several hippocampal-dependent learning and memory tasks [13].

The fragmentary information on the PKC family members in spatial memory made us carrying out a non-sophisticated study determining PKC family members in the hippocampus of mice trained in the MWM and indeed, only PKC gamma could be linked to memory retrieval.

2. Methods

2.1. Ethics statement

Experiments were done under license of the federal ministry of education, science and culture, which includes an ethical evaluation of the project (Project: BMBWK-66.009/0036/BrGT-2006). Housing and maintenance of animals were in compliance with European and national regulations. All efforts were made to minimize animal suffering and the number of animals used.

2.2. Animals

A total of 18 male CD1 mice, 10- to 12-weeks-old, were used for the experiments. This mouse strain is kept in the University's breeding facility for long and there is abundant experience in terms of behaviour. Mice were bred and maintained in cages made of Makrolon and filled with autoclaved woodchips in the Core unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna. Behavioural tests were performed between 8 am and 1 pm. During the experiments animals were individually housed.

2.3. Morris water maze (MWM) studies

The water maze used was a plastic, circular pool as described in detail previously [14–16]. The spatial acquisition phase consisted of four training trials per day (intertrial interval: 10 min) at four training days.

During the experiments animals were treated in groups of four. For each group, the target quadrant, where the platform was located, was randomly assigned and remained unchanged throughout the whole experiment. On the first training day, mice were given an acclimatization training session in the water maze as described before by Sunyer et al. [17]. Mice were released randomly with their heads facing the pool wall from the four compass locations (NE, NW, SW, and SE) and allowed to swim and search for the hidden platform for 120 s. If the mouse did not reach the platform within 120 s, it was guided to the platform. The time to reach the platform was recorded as the training latency for each trial.

Retrievals were carried out 7 days after the last training session to rule out the training effect and the effect from intensive handling. During retrieval a single probe trial was performed. Each mouse was released opposite of the platform quadrant without the platform into the pool and allowed to swim for 60 s. The probe trial was carried out for a shorter time to avoid the generation of depressive behaviour and to counteract memory extinction [18]. Time spent in the target quadrant and individual other quadrants was recorded [16]. Time spent in the target quadrant was compared to the average time spent in the three other quadrants. 9 trained and 9 untrained animals were sacrificed 6 h following the probe trial. Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until used for analysis.

Untrained controls were placed in the MWM to swim the same amount of time as their trained partners, but without a platform being present to climb onto. Since the animals were exposed to the same spatial cues, but without an escape platform, mice did not develop an association between the extra-maze cues and the location of the platform.

2.4. Sample preparation for immunoblot (Western)

Sample preparation of mouse hippocampal tissue representing the 20,000 × *g* supernatant followed by ultrafiltration with a cut-off at 10,000 Da (exclusion) i.e. a cell lysate, was conducted as described elsewhere [19]. Individual mouse hippocampi were homogenized and suspended in 1.2 mL sample buffer (20 mM Tris, 7 M urea, 2 M thiourea, 4%, w/v CHAPS, 10 mM 1,4-dithiothreitol, 1 mM EDTA, 1 mM PMSF, 1 tablet Complete™ from Roche Diagnostics, and 0.2%, v/v phosphatase inhibitor cocktail as given above). The suspension was sonicated on ice for approximately 30 s and centrifuged at 15,000 × *g* for 120 min at 12 °C. Desalting was carried out with an Ultrafree-4 centrifugal filter unit with a cut-off molecular weight of 10,000 Da (Millipore, Bedford, MA, USA) at 3000 × *g* at 12 °C until the eluted volume was about 4 mL and the remaining volume reached 100–200 μL [20]. The protein content of the supernatant was determined by the Bradford assay [21].

2.5. Western blot

Samples prepared as described above were loaded onto Excel-Gel SDS homogenous gels, using percentage shown in Table 1 (GE Healthcare, Buckinghamshire, UK). Electrophoresis was performed with the Multiphor II Electrophoresis System (Amersham Pharmacia Biotech, Uppsala, Sweden). Proteins separated on the gel were transferred onto PVDF membranes. Membranes were incubated with diluted antibodies as shown in Table 1. The IRDye-conjugated secondary antibodies used was goat anti-rabbit IgG (LI-COR, Lincoln, NE, USA, cat. 926-68021) in the same

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