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Research report

Intracerebroventricular injection of beta-amyloid in mice is associated with long-term cognitive impairment in the modified hole-board test

Sebastian Schmid*, Bettina Jungwirth, Verena Gehlert, Manfred Blobner, Gerhard Schneider, Stephan Kratzer, Kristine Kellermann, Gerhard Rammes

Department of Anaesthesiology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany

HIGHLIGHTS

- Aβ 1–42 injection is associated with cognitive impairment of declarative memory.
- Aβ 1–42 injection does not alter emotional behaviour.
- Aβ 1–42 injection induces elevated levels of Caspase 3.
- Changes in levels of Caspase 3 suggest apoptosis as an important factor for the development of cognitive dysfunction.

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ABSTRACT

Background: The intracerebroventricular injection of beta-amyloid (Aβ) in mice allows the investigation of acute effects on cognitive function and cellular pathology. The aim of this investigation was to further characterize the time course of Aβ-induced cognitive and behavioural changes and to detect potential molecular mechanisms.
Methods: Cannulas were implanted in the lateral cerebral ventricle. 14 days after surgery the mice were injected with Aβ1-42 or phosphate buffered saline (PBS). Starting 2, 4 or 8 (PBS only 4) days after injection we evaluated cognitive and behavioural performance using the modified hole board test (mHBT). We determined tumour-necrosis factor alpha (TNF alpha) and caspase 3 by western blotting, on days 10, 12 and 16. Data were analysed using general linear modelling, Kruskall-Wallis and Mann-Whitney-U test.
Results: Aβ induced a decline in cognitive performance represented as an increased total number of wrong choices during the testing period from day 2–15 ($p < 0.05$). Behavioural parameters were comparable between mice treated with Aβ and PBS. There was no difference regarding TNF alpha levels between the groups. Compared to day 16 Caspase 3 levels were increased on day 10 ($p = 0.004$).
Conclusions: Application of Aβ in the lateral ventricle of mice is associated with cognitive impairment of declarative memory in the mHBT. There is no interference caused by altered behaviour. Therefore, it represents a valid model for acute Aβ-mediated neurotoxic effects. Although the exact mechanisms remain unclear, changes in levels of Caspase 3 suggest apoptosis as an important factor for the development of cognitive dysfunction.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder over the age of 65 and is responsible for large costs in the healthcare system [1,2]. One of the key factors in the pathophysiology of AD is the accumulation of beta-amyloid (Aβ) in the brain [3]. Aβ is produced by proteolytic cleavage of amyloid precursor protein (APP). A mismatch of increased production and decreased clearance is discussed as the major reason for its accumulation in the brain. As APP can be cleaved in various positions and several post-translational modifications have been identified, different subspecies of Aβ exist [4]. Whereas Aβ 1–40 is most prevalent in the brains of patients suffering from AD, the ability to accumulate and form oligomers is increased in Aβ 1–42 [5]. It is postulated that these oligomers are much more neurotoxic than monomers and therefore are responsible for the neurocognitive deficits [6].

As Aβ plays a major role in the cascade of events leading to AD several groups injected Aβ in different brain regions of labora-

* Corresponding author.
E-mail address: seb.schmid@tum.de (S. Schmid).

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tory animals, predominantly rats and mice, in order to generate a model of AD. In these models acute interactions between Aβ and the brain can be researched [7]. Besides induction of synaptic dysfunction there is also a direct cytotoxic effect of Aβ. Additionally, an increased rate of apoptosis is present in the development of AD and cell death is mediated by a cascade of caspases [8]. Although a lot of research has been performed in this field the exact underlying mechanisms remain unclear [9].

Besides the pathophysiology of AD one of the main research goals remains an effective and specific therapy, which is not available yet [10]. In order to investigate the efficacy of a new therapy in a preclinical model, a comprehensive cognitive and behavioural test-battery is needed. Several studies investigating cognitive and behavioural performance after intracerebroventricular (icv) injection of Aβ in the brain revealed cognitive impairment [11–14]. However, especially in mice these studies tested neurocognitive impairment immediately after the application of Aβ and just for a very short period [11,12]. It remains unclear at what time-point the mice develop a cognitive deficit, how it evolves over time and whether there are changes in the mice's behaviour like increased anxiety that interact with the testing.

Therefore, aim of this investigation was to further characterize the time course of cognitive and behavioural deficits following icv Aβ injection in mice. For this task the modified hole-board test (mHBT) represents an ideal tool. In contrast to other tests that are mainly specialized on one or two parameters of cognitive or behavioural impairment it combines cognitive and behavioural parameters in one single test over several days [15]. Especially deficits in declarative memory can be detected which are common also in patients suffering from AD [16]. In order to determine the time when the mice start to develop cognitive deficits, the mHBT was performed starting on three different time-points after the icv injection. As secondary objective we analysed potential molecular mechanisms leading to the cognitive impairment.

2. Methods

The following experimental procedures on animals were approved by the Governmental Animal Care Committee (Regierung von Oberbayern, Maximilianstr. 39, 80538 Munich, Germany, Chair: Dr. B. Wirrer, Registration number: 55.2-1-54-2532-111-12, 27 November 2012).

2.1. Surgical procedure: implantation of intracerebroventricular cannula

Male C57BL/6 mice obtained from Charles River Laboratories (Sulzfeld, Germany) were housed under standard laboratory conditions (12 h light/12 h dark, 22 °C, 60% humidity and free access to water and standard mouse chow) 14 days prior to the experiments for acclimatisation.

The mice were injected intraperitoneally with a combination of midazolam (0.5 mg/kg), medetomidine (5 mg/kg) and fentanyl (0.05 mg/kg) for induction of anaesthesia. After loss of reflexes they were placed on a warming pad (rectal temperature was measured and maintained at 37.5 °C) and the stereotactic frame was mounted. The skin was shaved, disinfected and after local anaesthesia with xylocaine 2% a midline incision was performed to expose the scalp of the brain. Using a computer controlled motorized stereotactic instrument the insertion point of the cannula (1 mm lateral and 0.3 mm caudal of Bregma) was determined and a small hole (0.8 mm) was drilled. The cannula was placed with a depth of 3 mm using the stereotactic instrument. For further stabilisation a small screw was placed in the scalp and the cannula was cemented to the scalp and the screw. The skin was closed up using single stitches. Anaesthesia was terminated with injection of atipamezole (2.5 mg/kg), flumazenil (0.5 mg/kg) and naloxone (1.2 mg/kg) and the mice were placed under a warming lamp and monitored until full recovery. Afterwards the mice were placed in single cages and received 200 mg/kg of metamizol for pain treatment.

2.2. Injection of beta-amyloid

14 days after implantation of the cannula 18 mice were injected with Aβ 1–42 and 6 with phosphate buffered saline (PBS) through the cannula using a Hamilton® syringe connected to a plastic tube and a smaller cannula that was inserted into the icv cannula. 3.5 μl of PBS containing 1 μmol/l of Aβ 1–42 were injected followed by 1.5 μl of PBS. Before injection monomeric Aβ aliquoted using hexafluoroisopropanol and stored at −20 °C was warmed in a water bath at 37 °C for 10 min, then sonicated for 30 s, dissolved in NaOH (20 mmol/l, pH 12.2) and diluted in PBS to start the oligomerization process and sonicated for a further 30 s, mixed for 30 s, sonicated for 30 s and mixed one final time for 30 s before being placed on ice. The Aβ solution was used between 15 and 45 min following preparation and was brought to room temperature before use by loading into the cannula 10 min before administration.

2.3. Cognitive and behavioural testing

Starting on day 2, 4 and 8 after the injection of beta-amyloid and on day 4 after the injection of PBS the mice were tested using the modified hole-board test according to an established protocol. [15,17,18] In short: for the modified hole-board test the hole-board was placed in the middle of the test arena. Ten cylinders were staggered in two lines on the board. Each cylinder contained a small piece of almond, which was fixed underneath a grid and could not be removed by the animals. In addition, each cylinder was flavoured with the aroma of vanilla to attract the animals' attention. Three of the 10 cylinders were baited with a second – now approachable – piece of almond and marked with white tape. Testing was performed for 8 consecutive days until day 9, 11 and 15 after the intracerebroventricular injection. The animals were tested for four trials daily (300 s/trial). The sequence of marked holes was randomly changed every day. Deficit in overall cognitive performance was assumed if the time needed to complete one trial was extended (time complete), deficit within declarative memory was assumed if mice visited non-baited holes or did not visit baited holes referred to as wrong choice total, deficit within the visuo-spatial short-term memory was assumed if mice revisited a baited hole referred to as repeated choice. In parallel, several behavioural parameters were evaluated: *latency to first entry the board, board entries, immobility* and *time on the board* as indicators of avoidance behaviour, i.e. anxiety. The exploratory motivation, specified in: directed exploration, assessed by the *latency to the first hole visit, the total number of holes visited,* and general exploration, assessed by the number of *rearings*. The *physiological arousal* was determined by the time spent *grooming* and the number of *faecal and urine boluses*. The locomotor activity was assessed by the number of *line crossings*.

2.4. Sampling of brain and blood

On day 10, 12 and 16 after Aβ 1–42 icv application the brain of the mice was harvested by decapitation in deep anaesthesia and the samples were stored at −80 °C. One half of the brain was sliced into sagittal slides of 50 μm. The other half was separated into prefrontal motor cortex, sensor cortex and hippocampus.