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Research report

Blockade of hippocampal bradykinin B1 receptors improves spatial learning and memory deficits in middle-aged rats

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HIGHLIGHTS

- Increased B_1R expression in the hippocampus and cerebral cortex of middle-aged rats.
- Hippocampal B_1R antagonism improves the spatial learning and memory deficits of middle-aged rats.
- Hippocampal B_2R antagonism failed to reverse the cognitive deficits of middle-aged rats.

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ABSTRACT

Previous studies have demonstrated that targeting bradykinin receptors is a promising strategy to counteract the cognitive impairment related with aging and Alzheimer's disease (AD). The hippocampus is critical for cognition, and abnormalities in this brain region are linked to the decline in mental ability. Nevertheless, the impact of bradykinin signaling on hippocampal function is unknown. Therefore, we sought to determine the role of hippocampal bradykinin receptors B_1R and B_2R on the cognitive decline of middle-aged rats. Twelve-month-old rats exhibited impaired ability to acquire and retrieve spatial information in the Morris water maze task. A single intra-hippocampal injection of the selective B_1R antagonist des-Arg^9-[Leu^8]-bradykinin (DALBK, 3 nmol), but not the selective B_2R antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Hoe 140, 3 nmol), reversed the spatial learning and memory deficits on these animals. However, both drugs did not affect the cognitive function in 3-month-old rats, suggesting absence of nootropic properties. Molecular biology analysis revealed an up-regulation of B1R expression in the hippocampal CA1 sub-region and in the pre-frontal cortex of 12-month-old rats, whereas no changes in the B_2R expression were observed in middle-aged rats. These findings provide new evidence that inappropriate hippocampal B_1R expression and activation exert a critical role on the spatial learning and memory deficits in middle-aged rats. Therefore, selective B_1R antagonists, especially orally active non-peptide antagonists, may represent drugs of potential interest to counteract the age-related cognitive decline.

1. Introduction

Memory is vulnerable across the adult lifespan [1]. Remarkably, elderly subjects show a volume loss in forebrain regions that are important for learning and memory, such as the hippocampus and frontal cortex [2,3]. The complex gradual process of declining cognitive functions comprehends a large variety of molecular alterations in neuronal networks. Mitochondrial dysfunction, oxidative stress, autophagy, unbalanced calcium homeostasis and altered signal transduction pathways are examples of these alterations [4,5]. In addition, neuroinflammation is a common aging feature that affects hippocampal and cognitive functions [6,7].

The kallikrein–kinin system (KKS) plays an important role on inflammatory responses through the activation of bradykinin B_1 (by des-Arg^9-bradykinin and des-Arg^{10}-Lys-bradykinin), and B_2 (by bradykinin and Lys-bradykinin) receptors [8]. While the

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bradykinin B_2 receptor (B_2R) is constitutively expressed in many tissues under normal conditions, the bradykinin B_1 receptor (B_1R) is up-regulated during pathological states [9]. Specifically in the central nervous system, it has been suggested that B_1R exerts a critical role in inflammation-related conditions, including experimental autoimmune encephalomyelitis and Alzheimer's disease (AD) [10,11]. For instance, a sustained increase in B_1R expression in the hippocampus and pre-frontal cortex following amyloid-beta (Aβ) infusion in rodents was demonstrated [12,13]. Interestingly, genetic or pharmacological B_1R inactivation improves cognitive deficits observed in AD mouse models [13–15]. Moreover, a single bradykinin injection promotes learning and memory disturbance and also tau hyperphosphorylation in the rat hippocampus [16]. Remarkably, Lemos et al. [17] have demonstrated the B_1R genetic deletion improves 12 month-old mice spatial memory, while memory impairment was observed in age-matched wild-type mice.

Considerable amount of evidence indicates the crucial role of KKS in the memory loss associated with aging and neurodegenerative diseases. Despite, the specific role of KKS in the learning and memory processes on the hippocampus itself still is not well understood. Therefore, in the present study, we evaluated the role of hippocampal B_1R and B_2R in the cognitive function of middle-aged rats. In particular, we investigated the effects of selective B_1R or B_2R antagonists in the spatial learning and memory of 3- and 12-month-old rats. Selective antagonists were administered via intra-hippocampal and animals were evaluated on the water maze task. Additionally, we investigated putative age-related changes on B_1R and B_2R expression in the hippocampus and pre-frontal cortex of 3- and 12-month-old rats by immunohistochemistry and western blot.

2. Materials and methods

2.1. Animals

Experiments were conducted using 3- and 12-month-old male Wistar rats supplied by the animal facility of the Universidade Federal de Santa Catarina (UFSC, Florianópolis, Brazil). The animals were divided in three batches for behavioral (n = 8-10 animals per group), neurochemical (n = 5 animals per group) and immunohistochemical (n = 4 animals per group) studies. Rats were kept in collective cages (2–3 animals per cage) and maintained in a room under controlled temperature (23 ± 1 °C) and 12-h light cycle (lights on at 7:00 A.M.), with free access to food and water. All efforts were made to minimize the number of animals used and their suffering. The procedures used in the present study complied with the UFSC Ethics Committee on the Use of Animals guidelines for animal care, which follows the "Principles of laboratory animal care" from NIH publication No. 85-23.

2.2. Drugs

The selective B_1R antagonist des-Arg^9-[Leu^8]-bradykinin (DALBK, 3 nmol) (Sigma Chemical Co., St. Louis, MO, USA) and the selective B_2R antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Hoe 140, 3 nmol) (Aventis, Frankfurt Main, Germany) were prepared as 10^{-3} M stock solutions in phosphate-buffered saline (PBS) (pH 7.4) in siliconized plastic tubes. Stocks were maintained at −20 °C and diluted to the desired concentration just before the use. Bradykinin receptor antagonists doses were selected according to previous literature [13,18] and on pilot experiments (data not shown).

2.3. Stereotaxic surgery

Rats were deeply anesthetized with a 2:1 mixture of ketamine (75 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.) placed in a stereotaxic apparatus (Kopf, model 957) where bregma and lambda were kept on the same horizontal plane. A hole was drilled in the skull and bilateral stainless steel guide cannulas (23 gauge, 10 mm long) were lowered aiming at both dorsal hippocampus. The following stereotaxic coordinates from bregma were used according to the rat brain atlas: LL = ±2.8, DV = −2.2 and AP = −3.6 mm [19]. Two screws were implanted into the skull and fixed with dental acrylic. Two 30-gauge stainless steel stylets were placed into each guide cannula to prevent foreign materials entry. Seven days after surgery we performed intra-hippocampal infusions of vehicle (PBS) or the selective B_1R and B_2R antagonists followed by behavioral evaluation on the water maze.

2.4. Infusion procedures

We fitted two injectors (30-gauge, 11 mm long) into each guide cannula and applied dorsal hippocampus infusions using 10 μl micro-syringes (Hamilton, USA) attached to the injector by a polyethylene tube (PE10). Vehicle (PBS) or drugs (B_1R or B_2R selective antagonists) were injected at a 1.2 μl/min rate and 0.6 μl total injection volume using an automatic infusion pump (Insight, Brazil). Injectors were left in the place for additional 30 s after drug injection.

2.5. Water maze task

All behavioral experiments were carried out between 9:00 A.M. and 2:00 P.M. at least 2 h after animal habituation to the room. Same rater scored the animals and performed vehicle or drugs microinjections into dorsal hippocampus 5 min prior to the beginning of experiments. Sessions were monitored through a video camera positioned above the apparatus. An experienced experimenter blind to the treatments analyzed the videos using the ANY Maze® video tracking (Stoelting Co., Wood Dale, IL, USA). The water maze task was performed in a circular swimming pool similar to that described by Morris et al. [20]. Pool made of black fiberglass (1.7 m inside diameter/0.8 m high) was filled to a 0.6 m water depth maintained at 25 °C. Transparent Plexiglas platform (10 × 10 cm) was submerged 1–1.5 cm beneath water surface. Starting points trials were marked outside of the pool as north (N), south (S), east (E) and west (W). Four distant visual cues (55 × 55 cm) were placed on the walls of the water maze room. They were all positioned with the lower edge 30 cm above the upper edge of the water tank. The position of each cue marked the midpoint of a quadrant perimeter (circle = NE quadrant, square = SE quadrant, cross = SW quadrant and diamond = NW quadrant). Poll was located in a room with indirect incandescent illumination. A monitor and the video-recording system were installed in the adjacent room.

Animals were submitted to a spatial reference memory version of the water maze following a protocol previously described [21]. Training session consisted of 5 consecutive trials during which the animals were introduced into the tank facing the wall and then allowed to swim freely to the submerged platform. The platform was located in a constant position (middle of the southwest quadrant), equidistant from the center and the wall of the pool. If the animal did not find the platform during a period of 60 s, it was gently guided to it. Animal was allowed to remain on the platform for 10 s after escaping to it and then was removed from the tank. After 20 s animal was placed at the next starting point. Starting point (the axis of one imaginary quadrant) varied in a pseudo-randomized manner during the 5 sessions. Latencies to find platform were measured in each training session. Test session was carried out 24 h after the last training session. Each rat was allowed to swim for 60 s (single probe trial) in the water maze without the platform. Time spent in the correct quadrant (i.e. where the platform was located in the