Behavioural Brain Research 222 (2011) 134–140

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Serotonin 5-HT6 receptor blockade reverses the age-related deficits of recognition memory and working memory in mice

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ARTICLE INFO

Article history:
Received 21 July 2010
Received in revised form 14 March 2011
Accepted 18 March 2011

Keywords:
Serotonin
SB-271046
Aging
Recognition memory
Working memory
Object recognition
Spontaneous alternation

ABSTRACT

Studies have shown that the blockade of 5-HT6 receptors (5-HT6R) can improve memory processes and reverse age-related spatial episodic like memory deficits. Since normal aging in the human is associated with a decline in episodic and working memory, we assessed the effect of the 5-HT6R blockade (SB-271046) on recognition memory (object recognition task) (a component of episodic like memory) in parallel to working memory (spontaneous alternation task in the T-maze) performances in young, adult, aged and senescent mice. Deficits in consolidation of non spatial recognition memory that were observed in 17- and 21-month-old mice were found to be reversed by 5-HT6R blockade. Deficits in working memory performances were only apparent as late as at 25 months of age; again, these deficits were reversed by 5-HT6R blockade. This study revealed in the mouse that, as in humans, working memory is more lately altered than recognition memory during aging and that such memory deficits could be counteracted by the use of 5-HT6R antagonists.

1. Introduction

Normal and pathological aging are associated with a decline in cognitive performances such as learning and memory, with early alterations of episodic and working memory [8,25,27,28]. Although cholinergic and glutamatergic drugs are used for the symptomatic treatment of memory deficits in Alzheimer's disease (AD), there is a crucial need to discover new and efficient therapeutic strategies. In this context, serotonin receptors (5-HTR) represent promising therapeutic targets since the serotoninergic neurotransmission system is implicated in the modulation of learning and memory processes [7,46,53]. Among 5-HT receptors, 5-HT6 receptors (5-HT6R) are the most recently discovered 5-HT receptors. These receptors are G-protein-coupled receptors that stimulate cAMP synthesis [51,59] and are expressed in the rat and mouse central nervous system, notably in the cerebral cortex, *striatum*, *hippocampus*, *nucleus accumbens* and olfactory tubercles [4,26,30,32,56,65]. Such distribution suggests the implication of 5-HT6R in memory processes [2]. Accordingly, several studies undertaken in the past decade have strongly supported 5-HT6R as viable drug targets in the treatment of memory disorders associated with aging, AD and other pathologies. Indeed, in animal models, the blockade of 5-HT6R by selective antagonists improved various learning and memory processes (for review, [36]). Moreover, 5-HT6R antagonists were found to reverse scopolamine-induced deficits on recognition memory [33,40,49,52,61,69] and increase acetylcholine release in the *hippocampus* and frontal cortex [33,42,55,62,70]. However, a vast majority of these investigations were performed in young animals and only few works assessed the role of 5-HT6R in memory processes in aging and/or pathological animal models. Foley et al. [21] and Hirst et al. [33] respectively demonstrated that the chronic administration of SB-271046 (40 days) and SB-399885 (seven days), two selective 5-HT6R antagonists, reversed spatial learning deficits in the Morris water maze test in 20- and 22-month-old rats. In a recent contribution, we showed that the acute administration of SB-271046 was able to counteract the age-related deficit in the consolidation of spatial recognition memory (an episodic-like memory in rodents) in middle-aged and aged mice [11]. In parallel, Mitchell et al. [50] showed that chronic treatment with RO4368554 (14 days), another selective 5-HT6R antagonist, reversed non-spatial recognition memory deficits in middle-aged rats (15–18 months). Overall, these results strongly suggest that the 5-HT6R blockade seems beneficial in the restoration of episodic-like mem-

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doi:10.1016/j.bbr.2011.03.046

ory deficits associated with aging in rodents. Nevertheless, no study has yet assessed the putative implication of 5-HT6R on both episodic-like and working memory processes in young and aged rodents. Based on the role of 5-HT6R in memory modulation and the limited number of studies on the implication of 5-HT6R in episodic memory and working memory during aging, we were interested in this study to assess the effects of the blockade of 5-HT6R on both recognition memory and working memory through the use of the object recognition test and the delayed spontaneous alternation task in the T-maze, respectively, in young, middle-aged and aged mice. Such a paradigm was proposed to study both the putative deficit in recognition memory that could have been induced by a deficit in working memory and the effect of the 5-HT6R blockade on both types of memory. The memory involved in the one-trial object recognition task is that of an episode in the life of the animals, but this specific test assess only a particular aspect of this episode, i.e. the memory of the physical attributes of an object (what) [19]. An "episodic memory test" is concerned with a representation of the three constituents (what, where, when) of an event experienced in the past [19]. We thus considered the object recognition test, which is a commonly used and validated test [17,19], as a behavioural test allowing to assess, at least in part, some aspects of the episodic-like memory. Based on previous results [11,20] and the knowledge that aging is associated with learning impairments attributed to a deficiency of information consolidation in the *hippocampus* [23,66], the blockade of 5-HT6R has been induced by SB-271046 injected immediately after the presentation session of the recognition memory test. Since working memory deficits were still absent in aged mice (21 months), the study was extended to senescent mice (25 months) for working memory experiments.

2. Materials and methods

2.1. Animals

Experiments were performed on young (three/four months), middle-aged (10/11 months), aged (16/17 and 20/21 months) (sample sizes of 40 for each group of age) and senescent (25 months; $n = 15$) female NMRI mice (purchased at the age of three weeks from Centre d'Elevage René Janvier, Le Genest, France). Such a strain of mouse is commonly used in behavioural and pharmacological studies [29,54,64] and is a relevant animal model to evaluate the effects of a pharmacological modulation on learning and memory processes. Moreover, in comparison with other mouse strains, the life expectancies of NMRI mice is shorter, a feature that is interesting for practical reasons when one is implicated in aging studies. Interestingly, the age-related deficits in memory have been characterized in this strain of mouse by the group of Lamberty [29,37,38,39]. To limit putative biases induced by stress as observed when animals are housed alone for long periods of time (up to 25 months), we chose to house animals in small groups. In this respect, experiments were undertaken on female mice, since male mice cannot be housed in small groups for months because of their aggressive behaviour. In addition, studies demonstrated that recognition memory and working memory performances were similar between male and female mice of different strains during aging [3,34]. Animals were maintained in a controlled environment ($22 \pm 2\,^{\circ}C$; $55 \pm 10\%$ humidity) under a 12 h:12 h light/dark cycle (light on between 20:00 and 8:00) with food and water available *ad libitum*. Aged animals with obvious health problems (tumours, cataracts) were excluded before starting the experiments. All experiments complied with European Directives and French law on animal experimentation (personal authorisations No. 14-05 for FD, No. 14-17 for MB).

2.2. Drug administration

Mice received SB-271046 (5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophene sulphonamide hydrochloride), synthesised by Professor F. Fabis and Dr. M. Paillet-Loilier (CERMN, Caen), at a dose of 10 mg kg^{-1}. Previous studies have demonstrated that SB-271046 at this dose improved various forms of memory in mice and rats [11,22,32,35,41,43,57,65,68]. The drug was dissolved in 9‰ NaCl as the vehicle and administered i.p. at a volume of 10 ml kg^{-1}. Vehicle-treated animals were used for comparison.

2.3. Behavioural testing

One week before the beginning of the behavioural experiments, mice were handled daily by the experimenters. Behavioural tests were conducted during the dark phase of the cycle from 09:00 to 18:00. To calm the behaviour of the mice in the object recognition task and the spontaneous alternation test, pharmacological treatments were performed in a room adjacent to the one used for the behavioural test. Mice were placed in the treatment room 30 min before the beginning of the pharmacological treatments. After the treatments and behavioural tests, mice were replaced in their home cage.

2.3.1. Experiment 1: effects of the 5-HT6R blockade on recognition memory performance in young, middle-aged and aged mice

To assess the effects of the 5-HT6R blockade on recognition memory, the object recognition test was used. The test was performed in an open box (black-painted wood; 32 cm × 32 cm × 20 cm) with two types of objects available in four copies [blue dolphins (diameter 4 cm, height 10 cm) and sealed transparent bottles (3 cm × 4.5 cm × 9 cm) filled with sand] placed 5 cm away from the walls. In the experimental room, the light intensity provided from a halogen lamp directed at the ceiling was about 12 lx at the level of the animal. The procedure used to evaluate non-spatial recognition memory was based on Sik et al. [63]. Mice were familiarised to the apparatus (2 × 3 min) for three days (without objects) and tested on the fourth day. The task consisted of two sessions (3 min) separated by 60 or 240 min intertrial intervals (ITI). These two ITI were chosen on the basis of our previous study [11], which showed that aged mice that expressed spatial recognition memory deficits with ITI of 60 and 240 min in a place recognition test explored more the novel arm when treated with SB-271046 than with saline. Different cohorts of mice were employed for the 60- and 240-min ITI. During the task, a mouse was allowed to explore two identical objects during the training session, and then two dissimilar objects—an identical copy of the familiar and a novel one—during the retrieval session. From mouse to mouse, the role (familiar or novel object) and relative position of the two objects were counterbalanced and randomly permuted. The objects and the box were cleaned with diluted ethanol (70%) between each mouse. The time that the mouse spent sniffing (at a distance of no more than 1 cm) or touching (with their nose and forelimbs) the familiar and novel objects were collated (based on [63]). Mice were removed from the experiment (i) if they did not explore the familiar objects or only explore one of the two objects during the first session of the test and (ii) if mice did not explore the objects during the second session. The parameters measured were manually recorded (stop watches) by the experimenter who is located at one meter of the apparatus. Based on the study of Sik et al. [63], a discrimination index was also calculated ($[b - a]/[a + b]$, where a = exploration time of the familiar object and b = exploration time of the novel object). On the basis of our previous results [11], SB-271046 was administered immediately after the first trial to assess the effects of the 5-HT6R blockade on the consolidation phase.

2.3.2. Experiment 2: effects of the 5-HT6R blockade on working memory performance

2.3.2.1. Young, middle-aged and aged mice. To assess the effects of the 5-HT6R blockade on working memory, the delayed spontaneous alternation test in the T-maze was used. Spontaneous alternation is the innate tendency of rodents to alternate free choices in a T-maze over a series of successive trials [16]. Working memory is described in rodents as memory that allows the animal to memorise a delay-dependent representation of an object, location or stimulus which is used by the animal to realise a task [18]. Thus, in the T-maze, the ability to alternate between the two arms requires that mice retain a representation of a location at each trial that varies from trial to trial. The apparatus and experimental protocol used were similar to those described by Bontempi et al. [5]. The T-maze is made of transparent Plexiglas with a central arm (75 cm long × 12 cm wide × 20 cm high) and two lateral arms (32 cm long × 12 cm wide × 20 cm high) positioned at a 90° angle to the central arm. A start box (25 cm long × 12 cm wide) was separated from the central arm by a lever door. Sliding doors were placed at the entrance of each lateral arm. In the experimental room, the light intensity provided from a halogen lamp directed at the ceiling was about 25 lx at the level of the animal. Twenty-four hours before testing, mice were familiarised to the apparatus for 8 min. On the following day, mice were tested in a session of eight successive free trials in the T-maze. For each trial, mice were first placed in the start box for 30 s and then allowed to explore the maze and choose between the left and right lateral arm. After a 30 s confinement in the chosen arm, the mouse was removed and returned to the start box for the next trial. The maximum choice latency of 300 s was imposed at each trial. Urine and faeces were removed from the maze between trials, and the apparatus was cleaned with diluted ethanol (70%) between each mouse. The chosen arm was noted and the percentage of alternation over the eight trials was determined. This percentage was used as an index of working memory performance. Mice were treated with SB-271046 60 min before the first trial of the test. The 60-min delay was chosen based on the hypothesis that following i.p. administration, the peak concentration of SB-271046 in the blood would be obtained for a much shorter time, and clearance of the antagonist would be more rapid, than after p.o. administration (peak value at 180 min [58]).

2.3.2.2. Young and senescent mice. Since deficits in working memory were not observed in middle-aged and aged mice (see Section 3.2), we assessed working memory performance as described in Section 2.3.1 in older mice (25 months). A group of three-month-old mice were used as control animals. As previously, SB-271046 was administered 60 min before the delayed spontaneous alternation test in the T-maze.