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Behavioural Brain Research 83 (1997) 239–242

BEHAVIOURAL BRAIN RESEARCH

Research report

The effect of the loss of molar teeth on spatial memory and acetylcholine release from the parietal cortex in aged rats

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Received 23 August 1995; revised 8 March 1996; accepted 19 March 1996

Abstract

It has been demonstrated that a loss of teeth is a troublesome problem among age-related pathological phenomena of the oral cavity, which influences the entire body, due to the impairment of mastication. The present studies investigated the abilities of learning and memory and acetylcholine (ACh) release in the parietal cortex in aged rats without molar teeth (hereafter referred to as 'teethless'). After the molar teeth of rats were extracted, the rats were fed with powdered food for 135 weeks. Although the performance in the radial arm maze was progressively acquired by daily training, an increase in the number of errors and a decrease in the initial correct responses were observed in the teethless aged rats compared to the control aged rats, indicating impaired acquisition of spatial memory in the teethless aged rats. The basal level of extracelluar ACh in the parietal cortex was not different between the teethless aged rats and the control aged rats. However, the extracellular ACh level of the teethless aged rats under high-concentration of K^+ and atropine sulfate stimulation was significantly low compared to that of the control aged rats. These results suggest that the impairment of spatial memory in the teethless aged rats may be due to the functional deterioration of the cholinergic neuronal system induced by tooth loss and that there is a possibility that the loss of teeth may be one of the risk factors for senile dementia.

Keywords: Tooth loss; Aging; Acetylcholine; Spatial memory

1. Introduction

Geriatric studies have been shown to be very important as the aged population has rapidly grown in Japan. Diseases closely related with the elderly, so-called 'geriatric diseases', often become symptomatic after a long-term asymptomatic course. A complicated interaction of multiple factors is usually involved in the onset of these diseases, rather than a single cause. Thus, it is often difficult to clarify the mechanisms of onset and progression. While the geriatric dental medicine has been systematized in the field of clinical dentistry, fundamental studies on the relationship between aging and oral function have just taken the first step today. It has been demonstrated that age-related change in the oral environment can occur with both physiological and pathological aging. A loss of teeth is one of the most problematic changes in pathological aging. Furthermore, it has been demonstrated that the systemic effect of tooth loss is an epidemiological risk factor for Alzheimer's dementia [7]. Kawamura [6] has reported the relationship between mastication and learning and memory in young rats. He has also reported that rats fed with a powdered diet had poor results of learning and memory compared to those fed with a solid diet. Fujiwara [2] has reported the differences in neuronal density between the right and left cerebral hemispheres in rats with unilateral mastication.

In order to examine the effects of tooth loss on the central nervous system, we investigated the influence of the loss of molar teeth on the spatial memory in aged rats and on the release of acetylcholine (ACh) that plays an important role in learning and memory in humans and animals.

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PII S0166-4328(96)00126-X

2. Materials and methods

2.1. Materials

Male SLC Wistar rats (Nihon SLC Co. Ltd., Shizuoka, Japan) were used and were kept in a regulated environment (23±1°C, 50±5% humidity) with a 12-h light/dark cycle (light on between 09.00 and 21.00 h) and had free access to food and water.

2.2. Surgery

Animals (11 weeks old) were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and all maxillary and mandibular molars were extracted. Animals given anesthesia alone, without undergoing extraction of the molar teeth, were used as control aged rats.

2.3. Radial arm maze task

One hundred and thirty-five weeks after the surgery, the ability of learning and memory in the aged rats without molar teeth (hereafter referred to as 'teethless') was examined by using the radial arm maze [9], and compared to the control aged rats. The maze used in the present study consisted of 8 arms (48×12 cm) extending radially from a central area (32 cm in diameter), with a 5-cm edge around the apparatus [9]. The apparatus was placed 40 cm above the floor. At the end of each arm there was a food cup that held a single 50-mg food pellet. Prior to the maze task, animals were kept on a restricted diet and the body weight was reduced to 80–85% of their normal weight over a 1-week period; water was freely available. Before the actual training began, the animals were allowed to explore the apparatus, for 10 min a day, for 2 days. For the following 16 trials, each animal was placed individually in the center of the maze and allowed to consume the bait in the food cup. The training trial continued until all the bait in the food cup had been consumed or 10 min had elapsed. An arm entry was counted when all 4 limbs of the aged rats were within an arm. Re-entry into an already visited arm was regarded as an error. For each daily trial, the total number of errors was recorded. In addition, the number of initial correct responses defined as the number of successive correct responses made until an error was made, was also measured.

2.4. Measurement of extracellular ACh level using in vivo microdialysis

Nine weeks after the learning and memory study, the ability of releasing ACh in the parietal cortex of teethless aged rats was examined by using in vivo microdialysis methods [5]. The animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and placed in a stereotaxic apparatus. A guide cannula was implanted diagonally (30° angle against a cross-section) into the left parietal cortex according to the atlas (coordinates measured: A, −1.3 mm; and L, 3.4 mm from bregma; V, 2.9 mm from the skull surface). The animals were allowed at least 24 hours to recover following implantation of the guide cannula and then used in the microdialysis study. The location of dialysis probes was confirmed after each experiment.

Ringer's buffer (147 mM NaCl, 3.4 mM $CaCl_2$ and 4 mM KCl) containing 10^{-4} M physostigmine sulfate was perfused through the concentric microdialysis probe (3 mm) at a constant flow rate of 2 μl/min. The perfused dialysate was collected every 15 min. ACh in the dialysate was quantified by high-performance liquid chromatography (HPLC) system consisting of an immobilized enzyme reactor and electrochemical detector (ECD) (Eicom, Kyoto, Japan). The dialysate was separated by a column (Eicompak AC-Gel, 6×150 mm). The enzymatic reactor containing acetylcholinesterase and choline oxidase catalyzed the formation of hydrogen peroxide from ACh and choline. The resultant H_2O_2 was detected by ECD, with a platinum electrode at 450 mV. The mobile phase was 0.1 M sodium phosphate buffer (pH 8.5) containing 200 mg/l sodium 1-decanesulfonate and 65 mg/l tetramethylammonium chloride, with a flow rate of 1.0 ml/min. In order to examine the functional changes in cholinergic neuronal system of the teethless aged rats, animals were stimulated by high-concentration of K^+ at 100 mM or atropine sulfate at 3 μM for 15 min when the level of extracellular ACh stabilized.

2.5. Statistical analysis

Results are expressed as the mean±SEM. Comparisons between the control and the teethless aged groups were performed by analysis using Student's *t*-test. Statistical significance was defined as $P<0.05$.

3. Results

The control aged rats were fed pellet food and the teethless aged rats were fed the equivalent powdered food. The nutritional status was evaluated by regularly weighing the animals. We found that there was no weight difference between teethless rats fed powdered food and the control rats fed pellet food (data not shown).

Fig. 1 shows the number of errors in the radial arm maze task during training in the control and the teethless aged rats. The teethless aged rats showed impairment of performance during the acquisition of the radial arm maze task, as revealed by the increased number of errors (Fig. 1) and the decreased number of initial correct responses (data not shown).

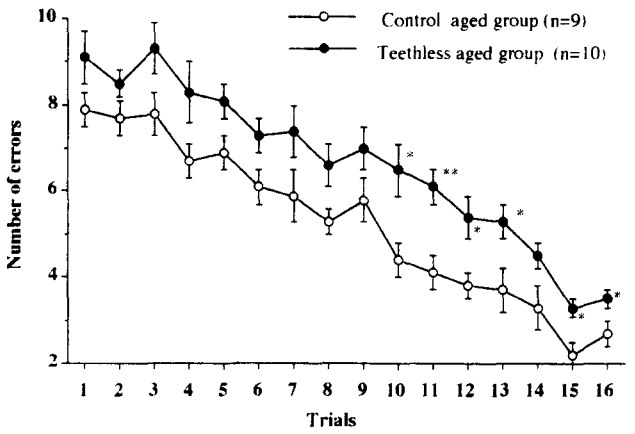

Fig. 1. Changes in the performance of aged rats without molar teeth (teethless) in the radial arm maze. Each point represents mean ± SEM. $*P<0.05$, $**P<0.01$ vs. control aged group.

As shown in Fig. 2, there were no significant differences in the basal level of the extracellular ACh in the parietal cortex between two groups. The maximum increase of the extracellular ACh level induced by the stimulation using high concentration of K^+ was 7 times larger than before stimulation in the control aged rats (Fig. 2). In the teethless aged rats, however, the degree of increase of the extracellular ACh level was significantly smaller than that in the control aged rats (Fig. 2).

The same phenomena were also observed by atropine sulfate stimulation (data not shown).

4. Discussion

In previous study, we demonstrated that the performance of teethless adult rats in a radial arm maze task is impaired [11]. In agreement with previous report, we found that the acquisition of spatial memory in the teethless aged rats was impaired, as indicated by the

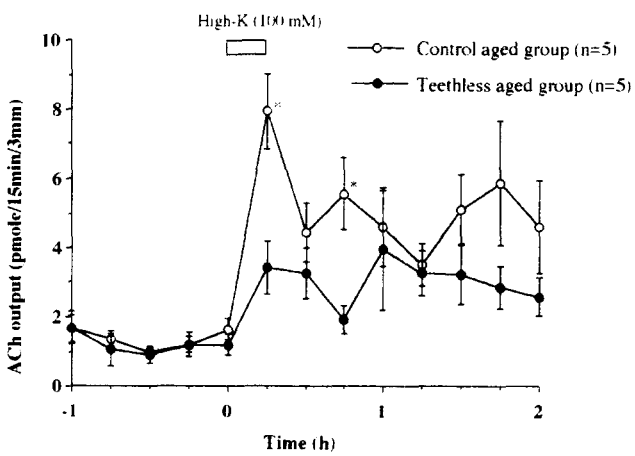

Fig. 2. Changes in the extracellular ACh level in the parietal cortex of aged rats without molar teeth (teethless). Each point represents the mean ± SEM. $*P<0.05$ vs. control aged group.

increased number of errors during daily training, compared to that of control rats. Kubota et al. [8] have reported that the periodontal membrane nerve completely disappeared from the extracted dental cavity and tiger-spotted lysis and clasmatosis of nerve cells in the trigeminal ganglion were observed 70 days after extraction of teeth in tupaidas and monkeys. Furthermore, the sensory input from the sensory receptor on the tooth root decreases as a result of long-term dysfunction of mastication caused by loss of teeth [8]. Abram and Hammel [1] and Rampone and Shirasu [10] have also reported that the muscular activity was increased by mastication, regardless of calorie intake, and the neuronal activity in the brain and cerebral blood flow simultaneously increased. Gobel [3] and Gobel and Binck [4] noted the primary neurons regulated by pulse of the secondary neurons in the spatial pathway of the trigeminal nerve were degenerated by extraction of hemilateral mandibular pulpectomy in cats. Thus, it could be inferred that the mastication-induced sensory stimulation in the pathway providing input from the proprioceptor of the periodontal membrane on the dental alveolar nerve to the midbrain nucleus via the trigeminal ganglion decreases in the teethless aged rats. From these findings, there is a possibility that loss of teeth produces neuronal dysfunction in the central nervous system, since mastication plays an important role in the function of the brain. Thus, it is suggested that the impairment of spatial memory in the teethless aged rats may be due to a decrease in mastication caused by the loss of teeth.

It is well accepted that the deficiency of learning and memory is partially related to selective loss or the degeneration of cholinergic transmission. In pathological aspects, Alzheimer's dementia and senile dementia of the Alzheimer type, which are characterized by progressive loss of memory and other cognitive functions, are associated with degeneration of cholinergic neurons. Taken together with these findings, the cholinergic neuronal system may play a very important role in learning and memory. In this study, thus, we measured the ability of extracellular ACh release in the parietal cortex of teethless aged rats using an in vivo microdialysis technique, in order to examine the functional relationship between learning and memory and cholinergic neuronal system: whether the impaired spatial memory in teethless aged rats is due to dysfunction of cholinergic neuronal system. In the present study, although the basal level of extracellular ACh in the parietal cortex was not different between the teethless aged rats and the control aged rats, the degree of increase of the extracellular ACh level induced by the stimulation of high-concentration of K^+ and atropine sulfate in the teethless aged rats was smaller than that in the control aged rats, suggesting the decrease in ability of releasing ACh in the teethless rats. As the hypothesis described above, thus, it is possible that impaired spatial memory in teethless aged

rats may be produced, at least in part, by the dysfunction of cholinergic neuronal system. The reasons for dysfunction of cholinergic neuronal system by the loss of teeth are not known. It has been demonstrated that the neuronal activity in the brain and the cerebral blood flow were increased by mastication [10]. Thus, one possible explanation may be that the dysfunction of cholinergic neuronal system in the teethless aged rats is caused by the long-term decrease of neuron activity of the brain and/or the cerebral blood flow by the loss of teeth. However, on this point, further investigation is required and other mechanisms involved in the effects of tooth loss remain to be elucidated.

In conclusion, we found that spatial memory and the cholinergic neuronal system were impaired by the loss of teeth, suggesting the possibility that any tooth loss would serve as a risk factor for dementia since it has been demonstrated that tooth loss is an epidemiological risk factor for Alzheimer's dementia [7].

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