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Motor and memory testing of long-lived pregnancy-associated plasma protein—A knock-out mice

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ABSTRACT

Mice deficient in pregnancy-associated plasma protein-A (PAPP-A), an IGF binding protein protease, have been shown to be resistant to experimentally induced atherosclerosis and diabetic nephropathy, and, in the laboratory environment, live 30–40% longer than wild-type littermates in association with delayed incidence and occurrence of age-related neoplasms and degenerative diseases.

Objective: PAPP-A is highly expressed in the cerebellum and hippocampus of the mouse brain. Therefore, the studies presented here were aimed at determining motor behavior, learning and retention in PAPP-A knock-out (KO) mice compared to wild-type (WT) littermates with age.

Design: Balance and coordination were assessed using an accelerating rotarod; learning and memory were assessed in a Stone T-maze.

Results: Time on the rotarod decreased with age but there was no significant difference between PAPP-A KO and WT mice at any of the testing ages. Latency to reach the goal box and number of errors committed in the Stone T-maze did not change with age and there were no significant differences between PAPP-A KO and WT mice.

Conclusion: Lack of PAPP-A in mice did not impact central regulation of coordination, learning or memory.

1. Introduction

Pregnancy associated plasma protein-A (PAPP-A) can modulate IGF-I action through cleavage of inhibitory IGF binding proteins [reviewed in 1]. Like IGF-I, PAPP-A displays antagonistic pleiotropy [2], i.e., expression is important early in life for optimal fetal growth and reproductive function but is associated in the adult with aging and age-related diseases [3,4]. We have shown that mice deficient in PAPP-A are resistant to the development of experimentally induced atherosclerosis, diabetic nephropathy, and visceral obesity [4–6]. Moreover, these PAPP-A knock-out (KO) mice live 30–40% longer than their wild-type (WT) littermates with delayed occurrence of spontaneous cancers and reduced incidence and severity of many degenerative diseases of age [7]. Therefore, PAPP-A has been proposed as a therapeutic target for aging and age-related diseases [8]. PAPP-A is expressed in the brain [3]; however, there are no data in the literature addressing possible function, positive or negative, of PAPP-A in the brain. Indeed, PAPP-A is highly expressed in the cerebellum and hippocampus of the mouse brain [9]. Therefore, the aim of this study was to determine whether the loss of PAPP-A in mice would impact central control of coordination, learning and memory.

2. Materials and methods

2.1. Mice

PAPP-A KO and WT littermates from heterozygous breedings were produced as previously described [3]. These mice are on a mixed C57BL/6, 129 genetic background. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of Mayo Clinic.

2.2. Rotarod

Mice were housed starting 1 month before and then throughout the period of testing in a shifted light:dark cycle to allow practical assessment of coordination during the start of the dark period when the mice are more active. In a preliminary experiment, we found that a large proportion of mice refused to stay on the rotarod during the light cycle and would rather sleep. An accelerating rotarod (RotaRod Advanced, TSE Systems, Inc., Chesterfield, MO) was used to measure overall balance and motor coordination of WT and PAPP-A KO mice at 4, 6, 12 and 18 months-of-age. The training period on day one consisted of placing mice on the rotarod revolving at a constant speed of 4 rpm for 3 min and then at 10 rpm for 3 min. On training days 2, 3 and 4 the mice

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were placed on the rotarod starting at 4 rpm with acceleration of 0.2 rpm/20 s up to 15 rpm for at least 3 min. Mice that failed training, i.e., no attempt to stay on the rod, were removed from the study. Only one PAPP-A KO mouse failed this training. The experiment was performed on the fifth day with the rotarod starting at 4 rpm and accelerating to 40 rpm at a rate of 0.2 rpm/20 s. The average latency to fall from the rotating rod during the testing period was calculated for each mouse. There were 12 mice in each group for testing at 4, 6, and 12 months. Two WT mice were lost to analyses at 18 months.

2.3. Stone T-maze

The dimensions and design of the Stone T-maze were as detailed in Pistell and Ingram [10]. Overall, the maze had black acrylic sides and a clear acrylic ceiling. It was constructed so that mice are required to wade, not swim, through water 2.2 cm deep and 20–24 °C to reach a dry, dark goal box. Thus, the Stone T-maze exploits a primary motivation of mice – escape to a safe location – in this case a location dry and dark. Mice first underwent straight-run training to establish the concept that moving forward would allow them to escape from the water and the light into the goal box. Any mice that were unable to reach the goal box in 15 s or less on 13 of 15 trials were excluded from further testing. Only one PAPP-A KO mouse failed the straight run. Acquisition trials in the maze were performed the next day, and consisted of 6 trials to learn the correct sequence of left and right turns to reach the goal box. In order to minimize hypothermia and fatigue, the entire group of mice was given a chance to complete the first trial before beginning the second trial. This allowed each mouse to have time between trials to rest and regain warmth. The primary measures of learning were the time to reach the goal box and the number of errors committed. An error was noted with the complete entry of a mouse's head into an incorrect path. During acquisition, if a mouse failed 3 times to reach the goal box within 3 min the trial was terminated and the mouse was removed from further analysis. Of the 22 WT and 17 PAPP-A KO mice in these studies, two WT mice and one PAPP-A KO mouse failed during acquisition. Retention was evaluated 1 week and 1 month following acquisition. Acquisition and retention were measured when mice were 6, 12, and 18 months. In one set of mice, acquisition and retention were only measured at 18 months. Also of note, three WT mice were removed from the study due to physical limitations (leg injury, severe kyphosis, morbid obesity) and one WT mouse died before the 18 month testing.

2.4. Statistics

Results are presented as mean ± SEM. Differences between WT and PAPP-A KO mice were evaluated by Student's *t*-test. Age- and time-related data were analyzed by repeated measures ANOVA. Significance was set at $P < 0.05$.

3. Results

3.1. Rotarod

Amount of time WT and PAPP-A KO mice spent on accelerating rotarod (latency to fall) is presented in Fig. 1. Similar results were obtained for males and females so data from the two sexes are pooled. A marked age-related decline (approximately 50% decrease between 4 months and 12 months) was seen in both WT and PAPP-A KO mice. Although there was a trend toward an increased latency to fall from the rotarod in older PAPP-A KO mice compared to WT mice, there was no significant difference between the two groups. Thus, young and old WT and PAPP-A KO mice did not appear to differ in their motor coordination.

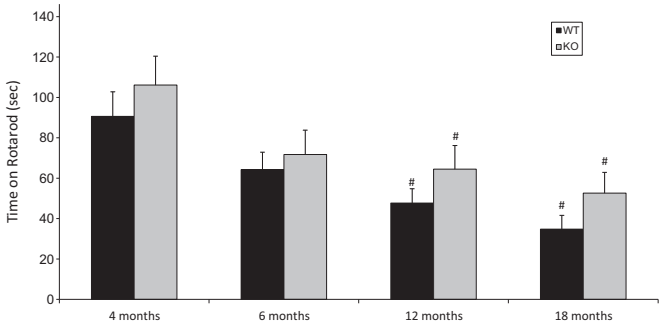

Fig. 1. Rotarod testing for balance and coordination of WT and PAPP-A KO mice. Results are mean ± SEM of 10–12 mice (two WT mice were lost to analyses at 18 months). #Significantly different from 4 months, $P < 0.05$. There were no significant differences between WT and KO.

3.2. Stone T-maze

The results from the Stone T-maze (latency, i.e., time to reach goal box, and number of errors per trial) for mice tested at 6, 12 and 18 months are presented in Figs. 2–4. Again, data from males and females are combined. At 6 months (Fig. 2), both WT and PAPP-A KO mice showed significant learning in the Stone T-maze, with approximately 50% decreases in run times and number of errors across acquisition trials. There were no significant differences between WT and PAPP-A KO mice in either latency or number of errors after 1 week following acquisition. After 1 month following acquisition, there were tendencies for PAPP-A KO mice to have decreased latency ($P = 0.08$) and reduced number of errors ($P = 0.06$) compared to WT littermates. With the same mice at 12 months (Fig. 3), acquisition of the maze information appeared faster than at the initial 6 months, and there were no significant differences in retention between WT and PAPP-A KO mice. At 18 months (Fig. 4), there appeared to be no further learning during the acquisition phase and no difference in retention between WT and PAPP-A KO mice. Furthermore, a separate group of 18-month-old mice that were not tested at younger ages also showed no significance difference between PAPP-A KO and WT mice in learning and retention in the Stone T-maze (Fig. 5).

4. Discussion

This study demonstrated that a lack of PAPP-A expression in the brain does not negatively impact motor coordination or learning and memory in mice. This is an important consideration in going forward with PAPP-A inhibition as a potential therapeutic approach to limit aging-related diseases and promote longevity.

Rotarod data were supportive of a previous study where metabolism and spontaneous motor activity were not different between 18-month-old WT and PAPP-A KO mice [11]. In this study, 4, 6 and 12-month-old mice were also included to evaluate any age-related changes in motor coordination. We found that rotarod performance declined by approximately 50% across age groups, similar to what has been seen in other studies of mice [12], but this decline was not affected in the absence of functional PAPP-A in the brain.

Despite age-associated decreases in motor function assessed by rotarod, mice at increased age did not have a significantly diminished performance level in the Stone T-maze. Furthermore, there were no significant differences between PAPP-A KO and WT mice in terms of latency to reach the goal box or number of errors. Acquisition trials demonstrated a clear ability of both groups of mice to learn, and trials after 1 week and 1 month indicated effective retention of the information. There were no significant differences between 18-month-old WT and PAPP-A KO mice whether they had undergone previous trials at earlier ages or were naive to the training. There are several advantages