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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.

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Conflict of Interest Statement

The authors and Mayo Clinic have no actual or potential conflicts of interest to disclose.

The data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at *Growth Hormone and IGF Research*.

There is a statement in the text that all animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of Mayo Clinic.

All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

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

Keywords

Pregnancy-associated plasma protein-A; Rotarod; Stone T-maze

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.

MATERIALS AND METHODS

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.

Rotarod

Mice were housed starting one 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 had 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 three minutes and then at 10 rpm for three minutes. On training days two, three and four the mice were placed on the rotarod starting at 4 rpm with acceleration of 0.2 rpm/20 seconds up to 15 rpm for at least three minutes. 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 seconds. 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.

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 seconds 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 minutes 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 one week and one month following acquisition. Acquisition and retention were measured when mice were 6, 12, and 18 months-of-age. 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.

Statistics

Results are presented as mean $\pm$ SEM. Differences between WT and PAPP-A KO mice were evaluated by Student t-test. Age- and time-related data were analyzed by repeated measures ANOVA. Significance was set at $P < 0.05$.

RESULTS

Rotarod

Amount of time WT and PAPP-A KO mice spent on accelerating rotarod (latency to fall) is presented in Figure 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.

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-of-age are presented in Figures 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 one week following acquisition. After one 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).

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 one week and one 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 naïve to the training. There are several advantages of the Stone T-maze for assessing age-related learning and memory, as discussed by Pistell et al. [13]. In particular, the Stone T-maze measures learning/memory independent of confounding factors arising from alterations in motor and visual function that occur in aged mice, including age-

related cataract development [14,15]. The Morris water maze, probably the most commonly used task for evaluating spatial learning and memory in rodents, is heavily dependent on visual ability and extra-maze cues [16].

We did not see the age-related decline in learning/memory that has been reported in WT mice [17–21]. There could be several reasons for this, including the use of mice with different genetic backgrounds, different tests especially those that rely on visual cues, and the fact that our 18 month-old mice are not ‘old’ although age-related differences are apparent at this age [3]. We chose 18 months as our oldest age group to avoid possible confounding variables, such as neoplasms especially in WT mice [3]. There were no significant differences between WT and PAPP-A KO mice at any of the ages tested, although there was a trend for decreased time and number of errors in the maze in 6 month-old PAPP-A KO compared to WT mice. An increase in the number of mice tested may provide greater statistical power. Others have reported improved cognitive ability in mice with extended lifespan due to caloric restriction or growth hormone deficiency/resistance compared to WT controls [17–21]. However, caloric restriction and growth hormone-resistant mice also show significant reduction in age-related cataract development [14,15], which could influence interpretation of the results.

The lack of an effect of PAPP-A deletion on these centrally controlled processes was somewhat unexpected since PAPP-A is highly expressed in the mouse brain [3,9]. However, PAPP-A is a modulator of local IGF action [1]. Circulating IGF-I appears to play a more important role in learning and memory [22], and PAPP-A KO mice have reduced local IGF-I signaling in the face of normal circulating levels of IGF-I. In addition, PAPP-A is particularly expressed in the CA1 region of the hippocampus [9]. This region is not as strongly associated with spatial memory as other areas, such as the dentate gyrus and CA3 region [23]. Therefore the Stone T-maze may not be the strongest indicator of deficiencies in this area.

Further studies are necessary to determine a role for PAPP-A in the brain, but the present study shows no major impact on coordination or cognition.

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HIGHLIGHTS

- Pregnancy-associated plasma protein-A (PAPP-A), a protease in the IGF system, is expressed in mouse brain
- Deletion of PAPP-A in mice did not impact balance and coordination (rotarod)
- Deletion of PAPP-A in mice did not affect learning and memory (Stone T-maze)

Figure 1.

Rotarod testing for balance and coordination of WT and PAPP-A KO mice.

Results are mean $\pm$ 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.

Figure 2.
Stone T-Maze: (A) Duration in maze and (B) number of errors for acquisition trials (left panel) and retention trials (right panel) for mice at 6 months.
Results are mean $\pm$ SEM of 8–10 mice.

Figure 3. Stone T-Maze: (A) Duration in maze and (B) number of errors for acquisition trials (left panel) and retention trials (right panel) for mice at 12 months. Results are mean $\pm$ SEM of 8–10 mice.

Figure 4.

Stone T-Maze: (A) Duration in maze and (B) number of errors for acquisition trials (left panel) and retention trials (right panel) for mice at 18 months.

Results are mean $\pm$ SEM of 8–10 mice

Figure 5.

Stone T-Maze: (A) Duration in maze and (B) number of errors for acquisition trials (left panel) and retention trials (right panel) for mice at 18 months.

Results are mean $\pm$ SEM of 6 mice