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CHRONIC INHIBITION OF MAMMALIAN TARGET OF RAPAMYCIN BY RAPAMYCIN MODULATES COGNITIVE AND NON-COGNITIVE COMPONENTS OF BEHAVIOR THROUGHOUT LIFESPAN IN MICE

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Abstract—Aging is, by far, the greatest risk factor for most neurodegenerative diseases. In non-diseased conditions, normal aging can also be associated with declines in cognitive function that significantly affect quality of life in the elderly. It was recently shown that inhibition of Mammalian TOR (mTOR) activity in mice by chronic rapamycin treatment extends lifespan, possibly by delaying aging {Harrison, 2009 #4}{Miller, 2011 #168}. To explore the effect of chronic rapamycin treatment on normal brain aging we determined cognitive and non-cognitive components of behavior throughout lifespan in male and female C57BL/6 mice that were fed control- or rapamycin-supplemented chow. Our studies show that rapamycin enhances cognitive function in young adult mice and blocks age-associated cognitive decline in older animals. In addition, mice fed with rapamycin-supplemented chow showed decreased anxiety and depressive-like behavior at all ages tested. Levels of three major monoamines (norepinephrine, dopamine and 5-hydroxytryptamine) and their metabolites (3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid) were significantly augmented in midbrain of rapamycin-treated mice compared to controls. Our results suggest that chronic, partial inhibition of mTOR by oral rapamycin enhances learning and memory in young adults, maintains memory in old C57BL/6J mice, and has concomitant anxiolytic and antidepressant-like effects, possibly by stimulating major monoamine pathways in brain.

Key words: mammalian target of rapamycin, memory, depression, anxiety, brain aging, monoamines.

INTRODUCTION

The target of rapamycin (TOR) is a major cellular signaling node that controls cellular metabolism and organismal lifespan in invertebrates and mammals (Kapahi and Zid, 2004). Mammalian TOR (mTOR) controls cell growth, proliferation, and survival through two distinct multiprotein complexes, mTORC1 and mTORC2. mTORC1 functions as a nutrient/energy/redox sensor and controls protein homeostasis. mTORC2 activates Akt/protein kinase B (PKB) by phosphorylation of Ser473 (Martin and Hall, 2005). This event inhibits the activity of FoxO transcription factors, which have a central role in the control of metabolism, cell stress resistance and autophagy (Michalek and Rathmell, 2008; Salih and Brunet, 2008). In addition, mTOR is involved in the modulation of long-lasting synaptic plasticity (Hoeffer and Klann, 2009). Although acute inhibition of mTOR has generally been associated with defects in long-term plasticity required for memory (Tang et al., 2002), inhibition of mTOR can also block the opposite process, the long-term reduction in synaptic responsiveness or long-term depression (LTD) (Huber et al., 2001). Moreover, disruption of signaling mechanisms that inhibit mTOR results in high mTOR activity and significant plasticity and memory deficits (Hoeffer and Klann, 2009). These observations suggest that mTOR does not act as a synaptic 'on-off' switch but may serve as a rheostat that modulates long-lasting synaptic change. The mechanisms by which mTOR inhibits memory have not been explored. Partial inhibition of mTOR function *in vivo* in rodent experimental models became possible only recently, when a method for effective chronic oral delivery of this drug was developed and used to establish that chronic systemic inhibition of mTOR in mice extends

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Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindolacetic acid; AD, Alzheimer's disease; DA, Dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EPI, Epinephrine; HPLC, high performance liquid chromatography; HVA, homovanillic acid; LTD, long-term depression; mTOR, mammalian TOR; NE, norepinephrine; NET, norepinephrine transporter; PKB, protein kinase B; PC, pars compacta; RN, raphe nuclei; TBS-T, TBS-Tween 20; TOR, target of rapamycin; VTA, ventral tegmental area.

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lifespan (Harrison et al., 2009). In agreement with these studies, we previously showed that treatment of mice modeling Alzheimer's disease (AD) fed with the same rapamycin-supplemented diet that extends lifespan blocked AD-like impairments in spatial learning and memory (Spilman et al., 2010). To explore the effect of chronic rapamycin treatment on normal brain aging we determined cognitive and non-cognitive components of behavior in C57BL/6J animals that were fed control- or rapamycin-supplemented chow at different ages throughout their lifespan, and for periods ranging from 8 to 40 weeks. Our results demonstrate that rapamycin treatment enhances cognitive function in young C57BL/6J mice and blocks age-associated cognitive decline in older animals. In addition, our data suggest that rapamycin has anxiolytic and antidepressant-like effects at all ages tested. Levels of three major monoamines (norepinephrine, dopamine and 5-hydroxytryptamine) were significantly augmented in midbrain of rapamycin-treated mice, suggesting that the effects of rapamycin on cognitive and non-cognitive components of behavior may be explained by the stimulation of major monoamine pathways in brain.

EXPERIMENTAL PROCEDURES

Mice

Mice used in these studies were C57BL/6J obtained from the Jackson Laboratories (JAX, Bar Harbor, ME, USA) or were non-transgenic mice arising from crosses of C57BL/6J breeders from JAX and heterozygous transgenic hAPP(J20) mice fully congenic in the C57BL/6J background as indicated in the figure legends and in the Results section. The twenty-five month-old C57BL/6 mouse group was purchased from Charles River. Numbers of animals per experimental group are indicated in the legends to the figures.

Rapamycin treatment

Mice were fed chow containing either microencapsulated rapamycin or a control diet as described by Harrison et al., 2009. Rapamycin was used at 14 mg per kg of food, verified by high performance liquid chromatography (HPLC). On the assumption that the average C57BL/6J mouse weighs 30 g and consumes 5 g of food/day, this rapamycin concentration in the chow results in an average dose of 2.24 mg rapamycin per kg body weight/day (Harrison et al., 2009). All mice were given *ad libitum* access to rapamycin or control food and water for the duration of the experiment. Body weights and food intake were measured weekly. Duration of rapamycin treatment for different groups is indicated in the Results section.

Passive avoidance

An inhibitory avoidance task was used to test long-term memory of an aversive event. Animals were habituated to the testing apparatus on day 1 by being placed in a lit chamber of a GEMINI active and passive avoidance system, a trough-shaped alley where two compartments (one lighted and one dark) are separated by a guillotine-like door. During the first 30 s (sec) of habituation the guillotine door was down. After 30 s the door was opened, allowing mice access to a similar but darkened chamber. At any point, mice that failed to enter the darkened chamber were taken out of the study. Twenty-four hours later mice were placed in the light side of the passive avoidance apparatus with the guillotine door down. After 30 s the door was opened allowing the mouse to cross to the darkened chamber. When the mouse crossed into the darkened chamber the door closed and an electric foot shock (2 mA for 2 s) was delivered through the grid floor. Mice were left in the chamber for 30 s before being returned to their home cage. Twenty-four hours later mice were again placed in the light side of the passive avoidance apparatus with the guillotine door down. After 30 s the door opened allowing the mouse to cross to the darkened chamber. The amount of time that it took for the mouse to cross from the light to the dark chamber (latency) was recorded. Each mouse had a maximum of 300 s to cross. No crossing was scored as 300 s.

Morris water maze (MWM)

The MWM (Morris, 1984; Galvan et al., 2006, 2008; Zhang et al., 2009) was used to test spatial memory. All groups were assessed for swimming ability before testing. None of the animals showed deficiencies in swimming abilities, directional swimming or climbing onto a cued platform during pre-training and had no sensorimotor deficits as determined with a battery of neurobehavioral tasks performed prior to testing. Briefly, mice were given a series of 3 trials per day, 20–30 min (min) apart in a tank filled with water opacified by the addition of non-toxic paint at a temperature of 24.0 ± 1.0 °C. Animals were trained to find a 12 × 12-cm submerged (1 cm below water surface) platform placed in one quadrant of the pool. The animals were released at different locations in each 60 sec trial. If mice did not find the platform in 60 sec, they were gently guided to it. After remaining on the platform for 20 sec, the animals were removed and placed in a dry cage under a warm heating lamp. The water tank was surrounded by opaque dark panels marked with geometric designs at approximately 30 cm from the edge of the pool that served as distal cues. The animals were trained for 4 days. At the end of training, a 30-sec probe trial was administered in which the platform was removed from the pool. The number of times that each animal crossed the previous platform location was determined as a measure of platform location retention. During the course of testing, animals were monitored daily, and their weights were recorded weekly. Performance in all tasks was recorded by a computer-based video tracking system (Water2100, HVS Image, Buckingham, UK). Data were compiled offline using HVS Image and compiled using Microsoft Excel before statistical analyses.

Tail suspension test

Mice were suspended from a wooden pole 45 cm above a procedure table using adhesive tape placed approximately 2.5 cm from the base of the tail. Periods of immobility and active movement were recorded for 5 min. Mice were considered immobile when they hung motionless.

Elevated plus maze

The elevated plus maze was used to assess emotionality and reactivity (Rodgers, 1997). The plus maze consists of two enclosed arms and two open arms. Mice are placed in the center of the maze and allowed free access to all arms for 5 min. The animals can spend their time either in a closed safe area (closed arms) or in an open area (open arms). While the open arms are, by design, anxiogenic, the animal is free to move into the closed arms. Mouse movements were recorded by a computer-based video tracking system (Maze2100, HVS Image). Time spent in the open and closed arms, distance