Original article

MOLECULAR METABOLISM

Peroxisome proliferator-activated receptor alpha plays a crucial role in behavioral repetition and cognitive flexibility in mice

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ABSTRACT

Background/objectives: Nuclear peroxisome proliferator activated receptor-α (PPAR-α) plays a fundamental role in the regulation of lipid homeostasis and is the target of medications used to treat dyslipidemia. However, little is known about the role of PPAR-α in mouse behavior.
Methods: To investigate the function of *Ppar-α* in cognitive functions, a behavioral phenotype analysis of mice with a targeted genetic disruption of *Ppar-α* was performed in combination with neuroanatomical, biochemical and pharmacological manipulations. The therapeutic exploitability of PPAR-α was probed in mice using a pharmacological model of psychosis and a genetic model (*BTBR T + tf/J*) exhibiting a high rate of repetitive behavior.
Results: An unexpected role for brain *Ppar-α* in the regulation of cognitive behavior in mice was revealed. Specifically, we observed that *Ppar-α* genetic perturbation promotes rewiring of cortical and hippocampal regions and a behavioral phenotype of cognitive inflexibility, perseveration and blunted responses to psychomimetic drugs. Furthermore, we demonstrate that the antipsychotic and autism spectrum disorder (ASD) medication risperidone ameliorates the behavioral profile of *Ppar-α* deficient mice. Importantly, we reveal that pharmacological PPAR-α agonist treatment in mice improves behavior in a pharmacological model of ketamine-induced behavioral dysinhibition and repetitive behavior in *BTBR T + tf/J* mice.
Conclusion: Our data indicate that *Ppar-α* is required for normal cognitive function and that pharmacological stimulation of PPAR-α improves cognitive function in pharmacological and genetic models of impaired cognitive function in mice. These results thereby reveal an unforeseen therapeutic application for a class of drugs currently in human use.

Keywords Lipids; Nuclear receptor; Ketamine; Memory; Neurodevelopmental disorders; Stereotyped behavior

1. INTRODUCTION

Dyslipidemia, a dysregulation of lipid content in the blood, is a leading risk factor for cardiovascular disease (CVD). Dyslipidemia is treated with fibrates, which act as agonists of the nuclear receptor peroxisome proliferator-activated receptor-α (PPAR-α) [1,2]. This protein is one of three PPAR isotypes, PPAR-α, PPAR-γ, and PPAR-δ, which display distinct physiological functions dependent on their differential ligand activation profiles and tissue distribution [3,4]. PPAR-α is abundantly expressed in skeletal muscles and liver where it increases lipoprotein lipase activity, increases production of enzymes associated with β-oxidation and augments levels of both ApoA-I and high density lipoprotein cholesterol (HDL-C) [5,6].
PPAR-α is also expressed in the brain, where its effects have not been as well characterized. In rodents, Ppar-α has been linked to brain dopamine function, a neurotransmitter system that is a target of some antipsychotic and autism spectrum disorder (ASD) medications. Specifically, Ppar-α activation improves antipsychotic medication adverse event oral tardive dyskinesia [7] and indirectly reduces the activity of dopamine cells in the ventral tegmental area in rodents [8]. In humans, dyslipidemia is more prevalent in individuals with schizophrenia and ASD compared to the general population [9,10], with one of the leading causes of premature death in these patients being CVD [1]. Several genetic association studies report an association between apolipoprotein E (ApoE), which plays an important role in lipoprotein metabolism and CVD, and schizophrenia [11]. Though individuals with schizophrenia and ASD may be at greater risk of dyslipidemia and subsequent CVD, the link between these conditions is not clear.

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Here we considered whether the cellular machinery that evolved to modulate energy availability also plays a neurodevelopmental role influencing cognitive behavior.

2. MATERIALS AND METHODS

2.1. Animals

For all experiments, male mice aged 3–5 months were used. No genotypic differences in body weight were evident at the time of testing. All procedures met the European guidelines for the care and use of laboratory animals (86/609/ECC and 2010/63/UE) and those of the Italian Ministry of Health (D.L. 116/92). Male wild-type and *Ppar-α −/−* (*B6.129S4-SvJae-Pparatm1Gonz*) mice previously backcrossed to *C57BL6* mice for 10 generations were bred in house, and the colony was established and maintained by heterozygous crossing. Mice were genotyped as described on the supplier webpage (http://jaxmice.jax.org), with minor modifications. DNA was extracted from tails using the RedExtract kit (Sigma–Aldrich, Italy).

BTBR T + Itpr3tf/J mice were purchased from Jackson Laboratory and a colony was maintained. All animals were housed in a 12-h light/12-h dark cycle with free access to water and standard laboratory chow diet. For all peripheral treatments, a volume of 10 ml/kg of solution was used.

2.2. Stereotaxic surgery

C57BL6 mice were injected with buprenorphine (0.05 mg/kg^{-1}, SC) 30 min before surgery and then anesthetized using a mixture of ketamine and xylazine (100 + 10 mg/kg^{-1}, IP). Mice were prepared for surgery, placed in a stereotaxic apparatus (Kopf Instruments) and a small incision was made in the skin above the skull. A drop of a pharmaceutical H_2O_2 solution was placed on the skull to increase visualization of bregma and lambda. A small hole was carefully drilled into the skull and a guide cannula (2.0 mm, 26G, Plastics One) aimed at the lateral ventricle (+1.0 mm (lateral), −0.6 mm (posterior) from bregma) was implanted using atlas coordinates (Paxinos and Franklin, 2001) and secured using dental cement. For ICV treatment, 3 μl of vehicle or drug was injected into the lateral ventricle over 1 min, using a 5–10 μl Hamilton syringe connected to a calibrated polyethylene tube. Cannula placement was confirmed at the end of the experiment through injection of methylene blue.

2.3. Behavioral assays

2.3.1. Novel Object Recognition (NOR) test

The NOR assay consisted of three testing sessions: a training session followed by two retention trials 15 min and 24 h later. Mice were habituated to the testing arena for two consecutive days before the test. During the training session, two different objects (A and B) were placed in the testing arena. Each animal was allowed to explore the objects for 5 min. The mouse was considered to be exploring the object when the head of the animal was facing the object or the animal was touching or sniffing the object. The total time spent exploring each object was recorded by a trained observer blind to treatment condition and expressed as percentage of total exploration time. In the retention sessions, one identical and one novel object (A and C or D) were used. A mouse was allowed to explore the objects for 5 min, and the time spent exploring each object was recorded. Exploration time was normalized as percentage of total exploration time. Preference for the novel object was considered as successful retention of memory for the familiar object.

2.3.2. Marbles burying test

20 small marbles were arranged in 5 × 4 rows in clean Plexiglas cages with fresh bedding (5 cm deep). Mice were introduced individually to this test arena under dim light and white-noise conditions. After 15 or 30 min, the mouse was removed and the number of unburied marbles was counted. A threshold of 75% coverage was used to determine whether the marbles were buried.

2.3.3. Self-grooming

Mice were manually scored for self-grooming behavior over a period of 10 min under dim light and white-noise conditions.

2.3.4. Spatial learning and memory tests

The Morris Water Maze (MWM) is a circular pool (diameter 170 cm, height 60 cm). The water temperature, 23 ± 1 °C, light intensity, external cues in the room, and water opacity were rigorously reproduced. A transparent Plexiglas non-slippery platform (diameter 10 cm) was immersed under the water surface (about 1.5 cm) during acquisition trails. Swimming was recorded using a camera coupled with video tracking software (Any-maze, Stoelting). Training consisted of 4 swims per day for 9 days, with a 15 min inter-trial interval. Start positions were pseudo-randomly selected and each animal was allowed a 60 s swim to find the platform. Once the mouse reached the platform it was allowed to remain on the platform for 15 s. The latency, expressed as mean ± SEM, was calculated for each training day. A probe test (60 s) was performed 24, 48 and 72 h after the last swim on day 9. The platform was removed, and each animal was allowed a free 60 s swim. The start position for each mouse corresponded to one of two positions remote from the platform location in counterbalanced order. The platform quadrant was termed the target quadrant and the percentage of time spent in the target quadrant was determined.

2.3.5. Reversal learning

A different cohort of mice was trained in the MWM apparatus as described above. On day 10, the platform was moved into a different MWM quadrant (see Figure 2G). The distance swum and the latency to find the new platform position together with the number of crossing on the previous platform position were recorded.

2.3.6. Spontaneous alternation performance

Each mouse, naïve to the apparatus, was placed at the end of one arm in a Y-maze (three arms, 40 cm long, 120 ° separate) and allowed to move freely through the maze during a single 5 min session. The series of arm entries, including possible returns into the same arm, was recorded visually. An alternation was defined as entries into all three arms on consecutive trials. The number of the total possible alternations was therefore the total number of arm entries minus two, and the percentage of alternation was calculated as correct alternations/possible alternations × 100.

2.3.7. NMDA antagonist-induced locomotion

Mice were allowed to explore the arena for 30 min, and their activity was monitored using a video tracking software (Any-maze, Stoelting). After 30 min, recording was paused, mice were injected with vehicle or treatment and immediately returned to the arena, and the video recording re-initiated. MK-801 was administered at a dose of 0.1 mg/kg^{-1}; SC and its effect recorded for 120 min. Ketamine was administered at a dose of 20 mg/kg^{-1}; IP and its effect recorded for 60 min.

2.4. EEG recordings

Ppar-α −/− and wild type littermates were chronically implanted with five electrodes under tiletamine/zolazepam (1:1; Zoletil 100®; 50 mg/

