Supporting information

Western blot and semiquantitative RT-PCR analysis

Samples from hippocampus and microvessels of mice were prepared, and immunoblot and RT-PCR assay were performed as described previously (Tang et al., 2014). For immunoblot, we used primary antibodies for rabbit anti-LRP1 (1:500), anti-RAGE (1:500), anti-NF-κB p65 (1:500), anti-PPARγ (1:500), anti-$A\beta_{1-40}$ (1:500), anti-$A\beta_{1-42}$ (1:500), anti-pro- or cleaved caspase-3 (1:500), anti-Bcl-2 (1:500), anti-Bax (1:500), anti-β-actin and anti-Histone H3 (inner control, 1:2000). The conditions for RT-PCR assay were as below: 94°C for 1 min, followed by 33 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 1 min, with a final extension step of 72°C for 7 min. The abundance of transcripts in cDNA samples was measured by RT-PCR，and the sequences of primers were followed: mouse RAGE forward 5'-ACATGTGTGTCTGAGGGAAGC-3' and reverse 5'-AGCTCTGACCGCAGTGTAAAG-3' (254 bp, Nucleotides 956-1169 in L33412, GeneBank), LRP1 forward 5'-GACCAGGTGTTGGACACAGATG-3' and reverse 5'-AGTCGTTGTCTCCGTCACACTTC-3' (299 bp, Nucleotides 2958-3099 in NM_008512, GeneBank) , β-actin forward 5'-TCTTGGGTATGGAATCCTGTG-3' and reverse 5'-ATCTCCTTCTGCATCCTGTCA-3' (154 bp, nucleotides 876-1029 in NM_007393.3, GeneBank).

Nuclear extracts preparation

Nuclear proteins were extracted according to nucleoprotein extraction kit (Tang et al., 2014). Briefly, isolated brain microvessels were homogenized in ice-cold hypotonic buffer(1 ml hypotonic buffer contain 5 μl phosphatase inhibitor, 10μl phenylmethylsulfonyl fluoride and 1μl DL-dithiothreitol) and centrifugation at 4°C, 3,000 g for 5 min was followed. The precipitate was washed with hypotonic buffer and centrifuged at 4°C, 5,000 g for 5 min. Finally, 0.2 ml lysis buffer (this buffer is the same as the buffer in the first step) were added into the precipitate, cooled for 20 min and centrifuged at 4°C, 15,000 g for 10 min. The supernatant nucleoprotein was used to Western blot for assay of NF-κB p65, PPARγ and Histone H3 was used as a loading control.

Immunohistochemistry

Immunohistochemistry was performed using the strept avidin-biotin complex (SABC) immunohistochemistry kit following the manufacturer's instructions. Briefly, anesthetized mice were intracardially perfused with 0.1MPBS which contained5 U/ml heparin and the mouse brains were fixed in 4% paraformaldehyde for 18 h, followed by 30% sucrose for 24 h. After that, brains were embedded into optimal cutting temperature (OCT) compound (Tissue-Tek, Torrance, CA) and dissected on dry ice. OCT-embedded frozen brain tissue sections were cryosectioned at a thickness of 18 μm and subsequently mounted on glass slides. After washed with 0.1M PBS, the sagittal sections were treated with 3% H_2O_2 at room temperature for 10 min and subsequently washed with PBS (3 × 5 min, 37°C). Sections were treated with 0.3% Triton X-100 for 15 min and then blocked with 5% BSA for 1 h and then incubated with anti-RAGE (1:100), anti-LRP1 (1:100), anti-$A\beta_{1-40}$ (1:100), anti-$A\beta_{1-42}$ (1:100) primary antibody diluted in blocking solution containing 0.3% Triton X-100overnight at 4°C. Sections were washed in PBS (3 × 5 min, 37°C), and incubated with biotinylated mouse anti-rabbit IgG at 37°C for 20 min, washed in PBS (3 × 5 min, 37°C), and incubated with SABC at 37°C for 20 min. After a further 4 × 5 min wash in 0.1 M PBS, Diaminobenzidine

was used as the final chromogen at 37°C for detection of target proteins. After gradient dehydration (70% ethanol 5 min, 95% ethanol 5 min, 100% ethanol 2 × 5 min, xylene, 2 × 5 min),photomicrographs, which was got using a Nikon DS-Fi2 camera that was connected to a Nikon Eclipse Timicroscope, was analyzed using the Image-Pro Plus software version 6.0. The number of RAGE- and LRP1-positive vessels was determined in ten randomly selected discontinous fields and was expressed as percentage per square millimeter of section (% positive vessels) (Shibata et al., 2000). Similarly, quantification of total $A\beta_{1-40}$ and $A\beta_{1-42}$ in the hippocampus was also performed, followed by the $A\beta_{1-40}$- and $A\beta_{1-42}$-positive area to generate the ratio of $A\beta_{1-40}$ and $A\beta_{1-42}$staining to total hippocampus area (% area occupied) (Cirrito et al., 2005; Deane et al., 2003). The average of 12 sections per mouse was determined and all of the above analyses were performed in a blinded manner.

Morris water maze (MWM) test

MWM was conducted to determine memory function in a room with extra maze cues during daylight. The training paradigm of MWM is composed of 4 trials (maximum duration/trial 90 s, interval 15 min) each day for five consecutive days, containing visible invisible platform training (day 1-2) and invisible platform training session (day 3-5). The last day is a probe trial. MWM test was carried out in a water-filled tank (diameter 120 cm, height 50 cm) which contained water (25 ± 1 °C, height 30 cm) and a platform (diameter 9 cm) submerged 1.5 cm in the center of the target quadrant. The visible-platform training was employed to assess baseline differences in vision and motivation, during which the platform was cued with a mounted flag (height 5 cm). The hidden-platform version (no flag) was used to evaluate spatial learning and determine the retention of memory to find the platform. The animals were allowed to search for the platform for 90 s. Each trial ended until the mouse located the platform and stayed on the platform for 10 s. If the mouse didn't reach the platform within 90 s, it was manually guided to it. Following the training paradigm (day 1-5), the probe trial was conducted to allow animal to swim in the tank without platform for 90 s. The number of target crossings and percentage of time spent in the quadrant were recorded. Data (latency for each trial, speed, number of target crossings, percentage of time spent in the quadrant) were monitored by a video tracking equipment and processed by a computer equipped with an analysis-management system (Viewer 2 Tracking Software, Ji Liang Instruments, China).

Y-maze test

The Y-maze test was used to evaluate working and short-term memory, which contained 2 consecutive days (training and testing trials respectively) (Tang et al., 2013). In this study, the Y-maze apparatus consisted of three compartments (10 ×10 cm) connected with passages (4 × 5cm) at a 120° angle from each other which were made of black plastic. There are 3.175 mm stainless steel rods (8 mm apart) on the floor of the Y-maze as well. The first day was learning trial, each mouse was put in one of the compartments and allowed to explore freely for 5 min in order to habituate to both passage and compartment, and then the mouse can enter the next session. In the process of training, two of the compartments were provided with the electric shocks through the stainless steel grid floor. Meanwhile, the third one with light on was free from shocks. Each mouse was trained for 10 times. If the mouse entered the shock-free compartment and stayed for 30 s, this time was recorded as a correct choice. If the mouse entered the shock-provided compartment, it was gently guided to the shock-free compartment and stayed for 30 s. The second day was testing trial, each mouse was also tested for 10 times

according to the same procedures. The latency for entering the shock-free compartment and the numbers of correct choices out of 10 on day 2 were recorded manually.

Open field test

To evaluate the general locomotor activity of the mice, open field test was conducted in this study (Wang et al., 2014). The apparatus was constructed of a black-painted plastic plate whose length width and depth are 50 cm respectively and divided into 144 squares. Each mouse was put at the area's center lightly which is the original place, and its total traveling distance was recorded for 5 min by using ANY Maze video tracking. One operation should be attentive: when each trial was done, the plate should be cleaned with ethanol solution (70% v/v), then the next mouse was put at the open field's center after the plate was dried with paper towels.

References

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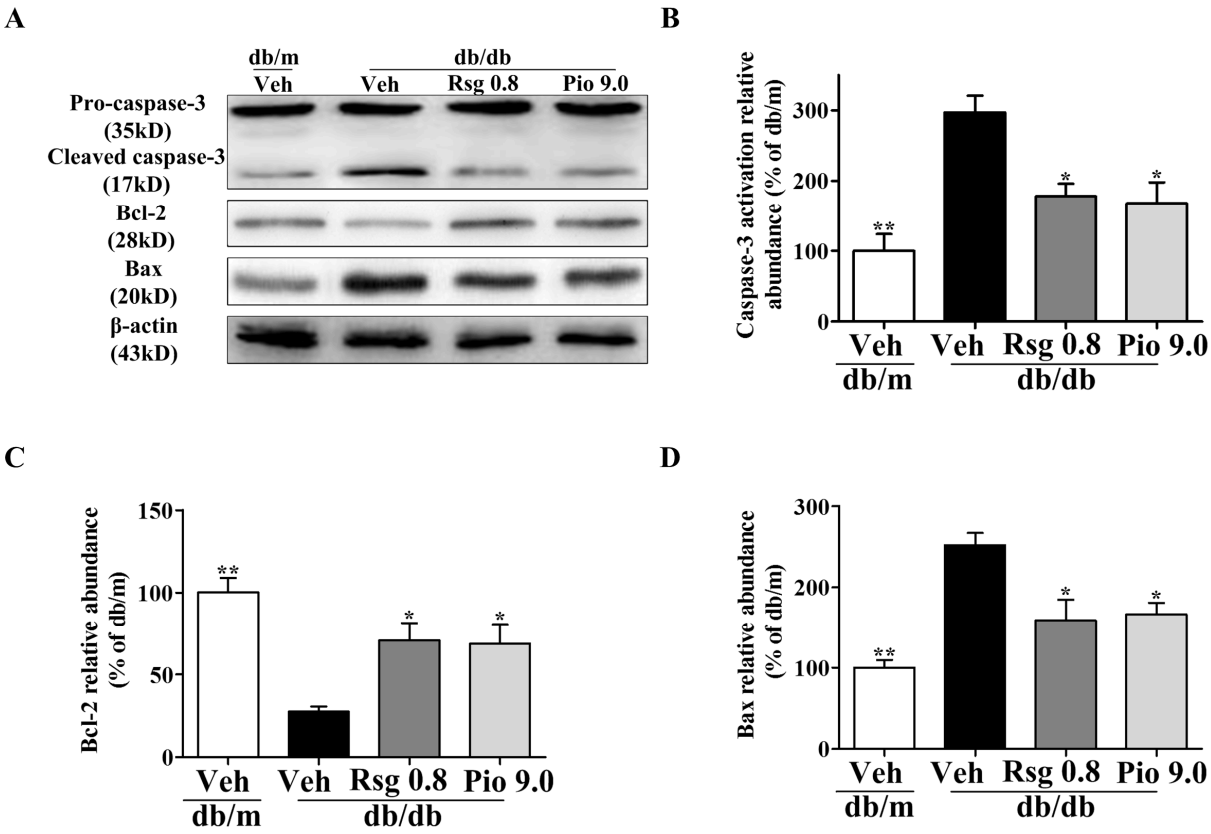
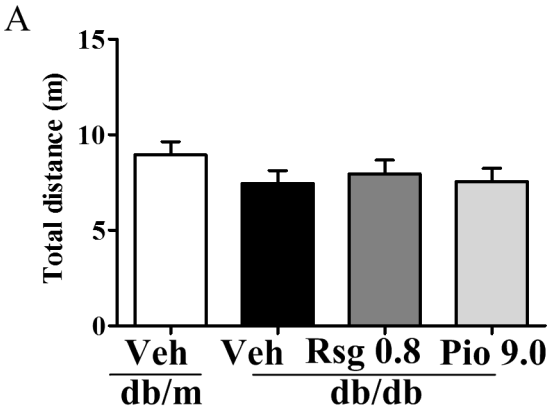


Supplementary Figure 1

Effects of PPARγ agonists on the levels of apoptosis-related molecules including caspase-3, Bcl-2 and Bax in hippocampus of *db/db* mice. (A) Pro-caspase-3, cleaved caspase-3, Bcl-2 and Bax were determined in hippocampus by Western blot using respective antibodies. (B) Caspase-3 activation is expressed as the ratio of caspase-3 fragment to pro-caspase-3, (C) Bcl-2 and (D) Bax were expressed as the ratio (in percentage) of the *db/m*. Values shown are expressed as mean ± SEM; n = 3. **P<0.05, **P<0.01 versus db/db+Veh* group.



Supplementary Figure 2

Effects of PPARγ agonists on the general locomotor activity in *db/db* mice. (A) The total distance traveled in the open field test is shown. Values shown are expressed as mean ± SEM;

n = 8-10.